

A Phase Contrast Study of the Haemocytes of the Larvae of *Spodoptera litura* (F).

BY

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ABSTRACT

Phase contrast microscope study revealed ten types of haemocytes viz., prohaemocytes, plasmatocytes, spindle form cells, vermiform cells, podocytes, cystocytes, spherule cells, granular haemocytes, genocytoids and adipohaemocytes in haemolymph of *Spodoptera litura* larvae. The total haemocyte counts ranged from 22,000 to 25,400 cells with a mean of 23,440 cell mm³/ of blood

INTRODUCTION

The haemolymph of insects can undergo quantitative changes to an extent virtually unknown for other tissues. In recent years increased attention has been paid to qualitative and quantitative studies of haemolymph. Recently, Jones (1962) made several suggestions for hematological analyses of insect blood and their application with a summary of current concepts concerning insect haemocytes. The study presented here is an attempt to characterise the qualitative and quantitative aspects of blood picture of the tobacco caterpillar *Spodoptera litura* (F.).

MATERIALS AND METHODS

Differential haemocyte counts (DHC): Final instar larvae of *S. litura*, 12-24 hours after ecdysing were used in the study. Haemocytes were studied in unfixed, undiluted, wet whole mounts of haemolymph from live larvae

and examined under phase contrast microscope after treating with versene, an anticoagulant. Minimum of 400 cells were examined for classification of different types of cells as suggested by Sarkaria et al. (1951).

Total haemocyte counts (THC): Haemocytes of ten larvae were estimated. The larvae were killed by immersing in hot water at 55°C to 60°C for 2-3 minutes. Blood was withdrawn by piercing the first pair of abdominal prolegs with a fine needle. The blood was collected in a Thoma white Blood Cell Pipette to the 0.1 mark, diluted with 1.5 per cent acetified saline solution to prevent clotting and made up to 11 mark. The blood cell counts were made by using double ruled Neubauer Haemocytometer. The number of haemocytes per mm³ of blood was calculated by counting the cells in five 1 mm square areas in each of the two chambers by using the formula given by Jones (1962) at a magnification of 450x,

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Haemocytes in five 1 mm² x Depth

factor x Dilution factor

Number of squares counted

where, depth factor = 10

dilution factor = 100.

The terminology suggested by Jones (1962) was adopted for classifying the various types of haemocytes of *S. litura*.

RESULTS AND DISCUSSION

The following ten cell types viz., prohaemocytes or proleukocytes, plasmatocytes, spindle form cells, vermiform cells, podocytes, cystocytes, spherule cells, granular haemocytes, oenocytoids, and adipohaemocytes were identified in the haemolymph of larvae of *S. litura* (Plate 1). The plasmatocytes constitute the major portion of the haemocytes counted, followed by prohaemocytes, spherule cells, granulocytes, genocytoids and adipohaemocytes. A brief account of the various haemocytes observed are furnished hereunder.

1. *Prohaemocytes* (Proleukocytes): Always small, mostly round to ovoid cells, generally with a relatively large, single centrally-located round nucleus (Plate I, Figs 1-2) and small amount of smooth cytoplasm. These cells are considered to be juvenile that will develop into plasmatocytes (Patton, 1963).

2. *Plasmatocytes*: Constitute the major portion of haemocytes, ovoid and disc-like with well defined relatively clear or granulated cytoplasm with

vacuoles and nucleus. They are exceedingly variable in form and tend to send out several to many fine, thread-like pseudopodia (Plate I, Figs. 3-5). Round spindle and irregular forms of plasmatocytes abound in the haemolymph.

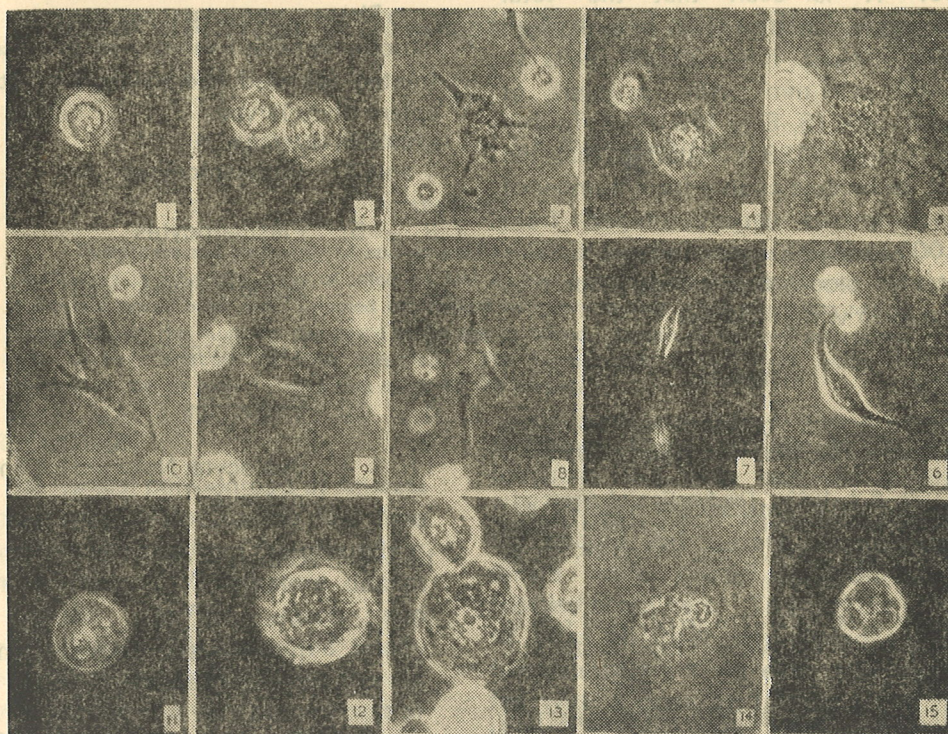
3. *Spindle shaped cells*: These possess small nucleus and well defined cytoplasm with vacuoles (Plate I, Fig 6.).

4. *Vermiform cells*: They are exceedingly long, thread-like forms resembling extremely elongated plasmatocytes and have small nucleus with large cytoplasm (Plate I, Fig. 7).

The spindle-shaped cells and vermiform type of cells now observed in *S. litura* may be nothing but the plasmatocytes which at times may send out pseudopodia or get rounded up or twisted at both ends as suggested by Jones (1962) and patton (1963).

5. *Podocytes*: *In vivo* observation indicated that plasmatocytes can send out pseudopods, which change them into podocytes. Podocytes are very large, extremely flattened plasmatocyte-like cells with three to eight or more long, rigid cytoplasmic extensions, (Yeager, 1945) (Plate I, Figs. 8-10). Rizki (1953) also contends that podocytes are only plasmatocytes with thread-like pseudopodia.

6. *Cystocytes*: Cystocytes are highly unstable haemocytes, all of which rapidly turn into brightly hyaline forms (Plate I, Fig 11) and cause granular clouds or glassy veils to appear

PLATE-1. Types of Haemocytes of *S. Litura* Larvae

in the plasma surrounding them (Jones, 1962). The nucleus is usually round and cartwheel-like; and is often extruded.

7. *Spherule cells*: Round or oval, have coarse spherules that practically occupy the whole cell (Plate I, Fig 12).

8. *Granular haemocytes*: Typically larger and noticeably thicker than plasmatocytes (Plate I, Fig 13). The granules generally tend to obscure the relatively small, round, centrally located nucleus in fresh material.

9. *Oenocytoids*: Only one type i. e., very large plasmatocytes-like cell, often occurring as bizarre variations of

the spindle form (Plate I, Fig 14) was noticed. These large cells are characterized by having extremely fine, long filaments at the spindle end, and by a large excentric nucleus often with two nucleoli. The inclusions are sometimes in the form of an irregular finely granular net work. The large bizarre oenocytoids often occur in clusters of two to four and in some appear fused to each other.

10. *Adipohaemocytes*: On rare occasions, haemocytes with an excentric nucleus and many brilliant fat-like droplets of various sizes have been encountered in the haemolymph (Plate I, Fig 15).

Total haemocyte counts

THC: It is seen that the total haemocyte counts (THC) of heat fixed larvae ranged from 22,000 cells to 25,400 cells/mm³ with a mean of 23,440 cells (S. E. \pm 113.83). Tauber and Yeager (1936) reported that the total haemocyte counts for a number of insect species usually ranged from 30,000 to 50,000 cells per cubic millimeter of blood, but the variation was large even within species.

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LEGEND. PLATE I, Figs 1-15

Phase contrast appearance of various types of haemocytes in versene treated, unfixed haemolymph of *Spodoptera litura* larvae.

- 1 and 3. Prohaemocytes
- 3 - 5. Plasmotocytes
6. Spindle shaped cell
7. Vermiform cell
- 8 - 10. Podocytes
11. Cystocyte. Note the excentric nucleus.
12. Spherule cell.
13. Granular cell. Note the numerous distinct small round granules.
14. Oenocytoid. Note the fine granular network in the cytoplasm and excentric nucleus.
15. Adipohaemocytes. Note the large lipid inclusions and the typical excentric nucleus.