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A Comparative Study of Haemocytes in Some Coleopterous Species

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Among four Coleopterous species, *Dermestes vulpinus*, *Dermestes maculatus*, *Hybosorus illigeri*, and *Dineutes aereus*, six morphological types of haemocytes are identified by a light microscope: prohaemocytes, plasmatocytes, granular haemocytes, oenocytoids, adipohaemocytes and spherule haemocytes. All six cell types are not present in every species. For example, adipohaemocytes and spherule haemocytes are not found in larval stages of *D. maculatus*.

The structure and occurrence of these haemocytes are described along with the structural variations which occur in each type. The cyclic changes in the insects' life cycle in the presence of different types of haemocytes are interestingly found to be related to their physiological stages.

Substantial advancement in the established knowledge of insect haemocytes is the result of the steadily increasing accumulation of recent research, due to the increased interest of workers involved in the subject and the development of new techniques (Arnold 1970; Gupta 1969, 1979; Jones 1962; Kayya and Ratcliffe 1982; Raina and Bell 1976) in view of the vital roles of these haemocytes in defense, storage, phagocytosis and their effective reactions to poisons (Jones 1965; Roeder 1953; Wigglesworth 1965).

In spite of the large amount of information available from a variety of insects, the very basic question of the classification and naming of haemocytes needs to be standardized and adopted by most of the scientists involved (Gupta 1979). Our intention in bringing out the following research is to report the early observations

of elaborate studies on the different physiological aspects of haemocytes to be carried out in the future.

The present study is also an attempt to follow reliable and acceptable nomenclature in accordance with the criterion for the identification of different types presented by Arnold and Hinks (1975), Gupta (1979), Jones (1962) and Kayya and Ratcliffe (1982), as well as some information about the changes in the differential haemocyte counts in relation to ecdysis (Tauber 1937), that may serve as an addition to the information required to reach a consensus in naming the insect haemocytes, particularly in Coleopterous insects.

Materials and Methods

Adult and larval stages of *Dermestes vulpinus* and *Dermestes maculatus* were collected from the outskirts of Al-Kharj, a district seventy kilometers to the south of Riyadh, generally from the area where sheep, goats and camels are slaughtered, especially from on and around the skin and waste of the butchered animals. A mixed colony of both species was maintained in the laboratory at 25 ± 2 °C and 70% relative humidity, and these were fed upon the skin of sheep.

Similarly, *Hybosorus illigeri* and *Dineutes aereus*, the aquatic beetles, were collected from ponds and irrigation canals from an area rich in decaying organic materials in water, from Hofoof, 325 kilometer east of Riyadh. These were maintained in the laboratory with a constant supply of oxygen in an aquarium with a temperature of 25 ± 2 °C which was adjusted with the aid of light bulbs. The colony of *H. illigeri* was fed on living chironomus larvae, while that of *D. aereus* was fed upon decaying leaves.

To study the effect of aging, specimens of the desired age and stage were made available for experimentation. A group of at least 200 haemocytes from each insect was counted for differential haemocyte counts. Five to ten insects were used in each experiment.

Insects were heat-fixed at 50 °C for two minutes prior to making them bleed. An antenna or foreleg of the insect was severed and the abdomen was gently pressed to facilitate the oozing of blood which was collected either on the slides for smearing or into a micro pipette for total haemocyte counts. Cytological details of the haemocytes were carried out: Giemsa's, Wright's, Lieshman's stains were used.

For the identification of different possible categories of haemocytes, several methods were attempted such as hanging drop preparations under phase contrast microscope (Carl Zeiss). For the study of the morphology, size, staining reactions and differential haemocyte counts (DHC), Giemsa's, Lieshman's, Wright's and Eosin stains were used.

Results

Morphological variations, staining reactions of the nucleus and cytoplasm, position and comparative size of the nucleus with respect to the size of the whole cell, and the size and location of the vacuoles in the haemocytes served as some of the important features of the criteria used to classify the different categories of haemocytes. Apart from this, the use of phase optics proved to be very useful for naming different categories.

To avoid the possibility of innovation, a parity to the standard terminology of Jones (1962) and Gupta (1979) was maintained. A maximum of six types of haemocytes were identified and labelled as prohaemocytes, plasmatocytes, granular haemocytes, oenocytoids, adipohaemocytes and spherulocytes.

Prohaemocytes

Prohaemocytes (PRs) are the smallest cells in all the species studied with no exception. Comparing their whole individual area, these cells have the largest nuclei, and consequently the lowest value of the ratio of the volume of the cell to the volume of the nucleus within the cell. These cells possess smooth cell membranes and nuclear membranes with nuclei inclined to the basophilic nature and a scanty amount of cytoplasm around the nucleus which remain comparatively acidophilic in all the four species of coleopterous insects (Figs. 1a, b, c; 2a, 3a, b and 4a and b).

PRs measure 6–10 μm in diameter when spherical, but sometimes these cells also attain an oval shape; their size ranges between 5–8 μm in width and 6–11 μm in length, retaining the strictly round shape of the nucleus (5–8 μm) (Fig. 1b and c).

In both *H. illigeri* (Fig. 3b) and *D. aereus* (Fig. 4b, i), PRs protrude into a threadlike projection and then somehow resume a spindle shape that does not exceed 11 μm. Among all four species, the nuclei in PRs appear to be thickly packed balls of chromatin granules.

The relative percentage of PRs ranges from 10% to 39%, and the maximum percentage is found in the adult stage of *D. vulpinus*, (Fig. 6b) that undergoes a decrease in the larval stage immediately after third ecdysis to just 10%. The trend of decrease just after ecdysis and increase before ecdysis in the PRs population is interestingly observed unanimously in all four species studied. The population of PRs suffered a loss in percentage and an increase thereafter until the next ecdysis. The addition to the population of PRs is recorded after emergence and continues until six days after emergence (Figs. 5c, 6c, 7c and 8c).

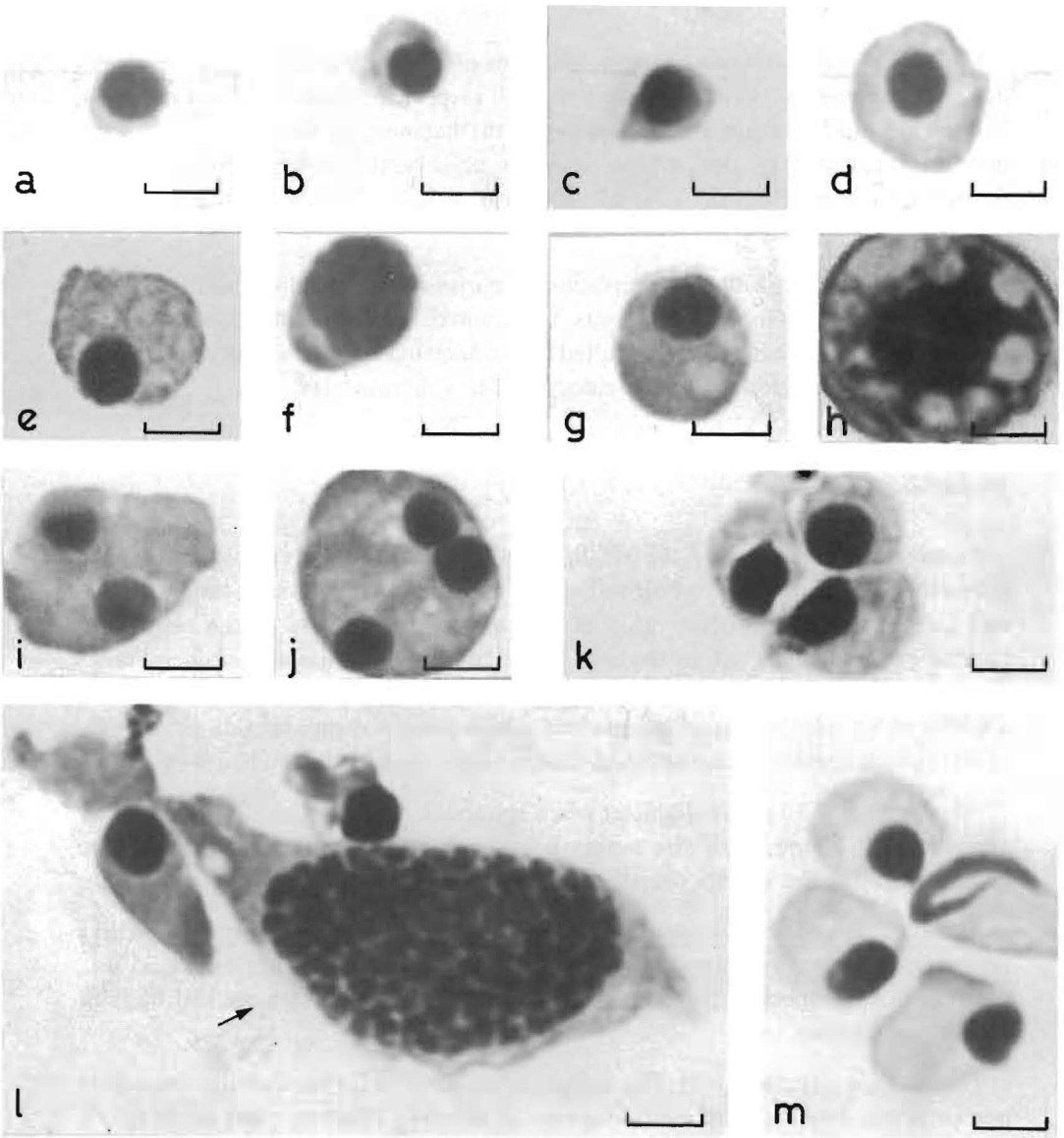

Fig. 1. A photomicrograph of representatives of different types of haemocytes in *Dermestes maculatus*: a, b, c—Prohaemocytes; d, k, l, m—Plasmatocytes; e, f-Granular haemocytes; g—Oenocytoids; h—Adipohaemocytes; i, j—Spherulocytes. (Scale bar = 10 μm.).

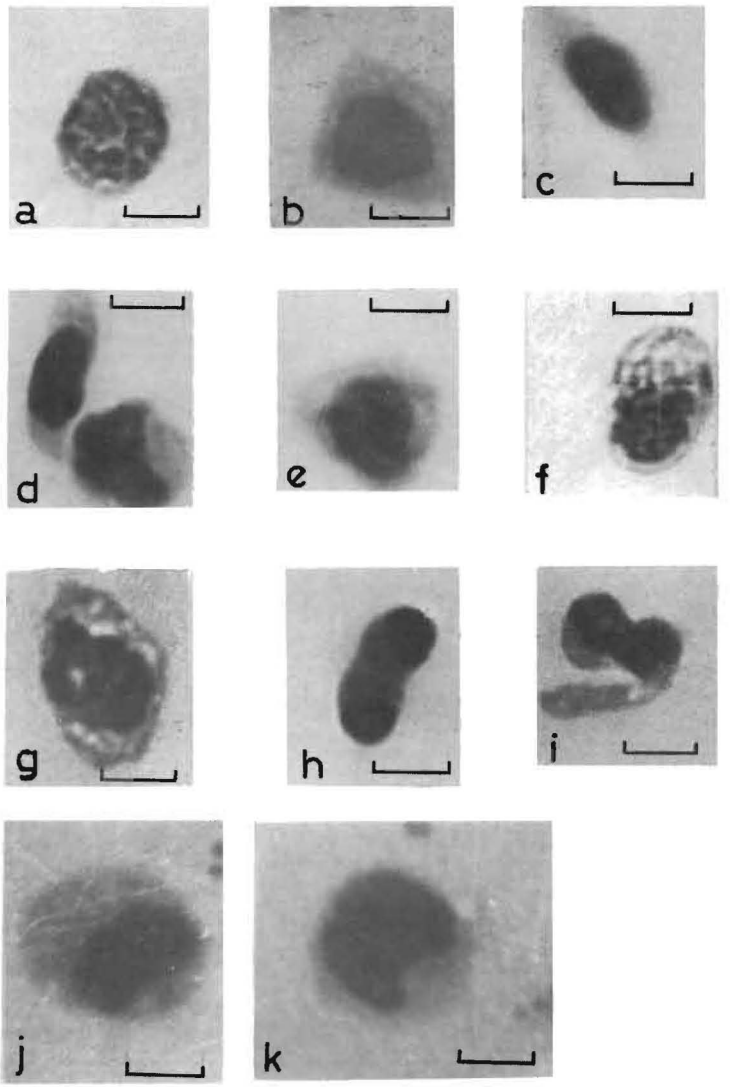

Fig. 2. **A photomicrograph of representatives of different types of haemocytes in *Dermestes vulpinus*: a—Prohaemocytes; b, c, d—Plasmatocytes; f—Granular haemocytes; j, k—Oenocytoids; g—Adipohaemocytes; e—Spherulocytes; h, i—Mitosis in Plasmatocytes. (Scale bar = 10 μm.).**

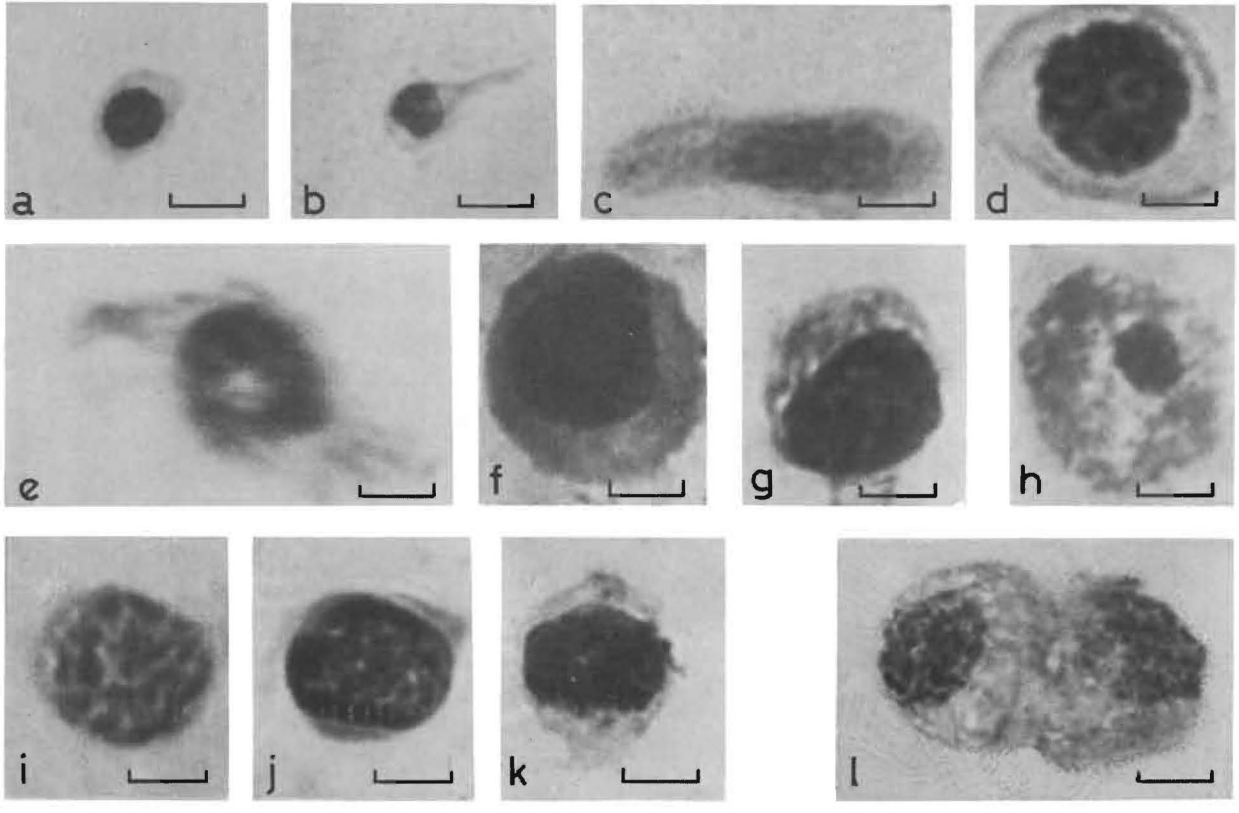

Fig. 3. A photomicrograph of representatives of different types of haemocytes in *Hybosorus illigeri*: a, b—Prohaemocytes; c, d, e—Plasmatocytes; f—Granular haemocytes; g—Oenocytoids; i, j, k—Adipohaemocytes; h—Spherulocytes; l—Mitosis in Plasmatocytes. (Scale bar = 10 μm.).

Plasmatocytes

The plasmatocytes (PLs) are generally larger cells with polymorphic nature with spherical, oval, spindle to fusiform and sometimes amoeboid shapes with a few small vacuoles and clear cytoplasm. Spherical shaped PLs are more prevalent except in *D. vulpinus* where the spindle shaped PLs occurred in higher numbers. The nucleus is much smaller in comparison to the size of the cell (Figs. 1d, k, m, l; 2b, c, d, e; 3c, d, e, and 4c, d, f, i, j, k, l).

Comparatively, the size of PLs of a similar shape is larger in *D. maculatus* (8–15 μm) (Fig. 1b) than in *D. vulpinus* (7–12 μm) (Fig. 2b, c). The round or spherical shaped PLs ranged from 10 to 20 μm in diameter in *H. illigeri* (Fig. 3c, d, e) and 6 to 11 μm in *D. aereus*. The oval shaped cells measured between 10–20 μm in width and 10–25 μm in length. The largest oval shaped PLs are found in *H. illigeri* (Fig. 4d) and the smallest in *D. maculatus* (Fig. 1c). The spindle shaped PLs measure 4–18 μm in width and 12–50 in length in all four species. However, the smallest spindle shaped PLs are identified in *H. illigeri* (Fig. 3b) and the largest in *D. maculatus* (Fig. 11).

Granular haemocytes

The granular haemocytes (GRs) are identified as almost round cells with the presence of several minute granules in their cytoplasm except in *D. aereus* where these cells assumed spindle shapes (Fig. 4f and 7). The occurrence of small vacuoles is noticed in lesser numbers than in round PLs. These cells failed to distinguish themselves from round PLs in Giemsa's stained preparations. GRs are easily identified with difference in hanging drop preparation under phase optics (Figs. 1e, f, 2f, 3f and 4f).

The size of PRs varies from 9 to 19 μm in all four beetles; the smallest size is noticed in *D. aereus* (Fig. 4f) and the largest in *H. illigeri* (Fig. 3f). Variation within each species ranged from 9–16 μm in *D. aereus* (Fig. 3d), 10–14 μm in *D. vulpinus* (Fig. 2e), 12–17 μm in *D. maculatus* Fig. 1e) and 10–19 μm in *H. illigeri* (Fig. 3f).

The nucleus in GRs is observed to be mostly round except in *D. vulpinus* (Fig. 2e), and *D. aereus*, where it assumes a slightly oval shape, the measurement 7–12 μm applies to all four species studied, and variation in the size of the nucleus is in accordance with the corresponding size of the cell.

The GRs are third in population in each species except in *D. aereus*, where their percentage exceeds the percentage of PRs; in this instance their percentage ranged from 6 to 19. Their minimum percentage is reported from *D. maculatus* three days before the third ecdysis and the maximum number is found in *D. aereus* three days after the third ecdysis (Figs. 5b, 6b, 7b, and 8b).

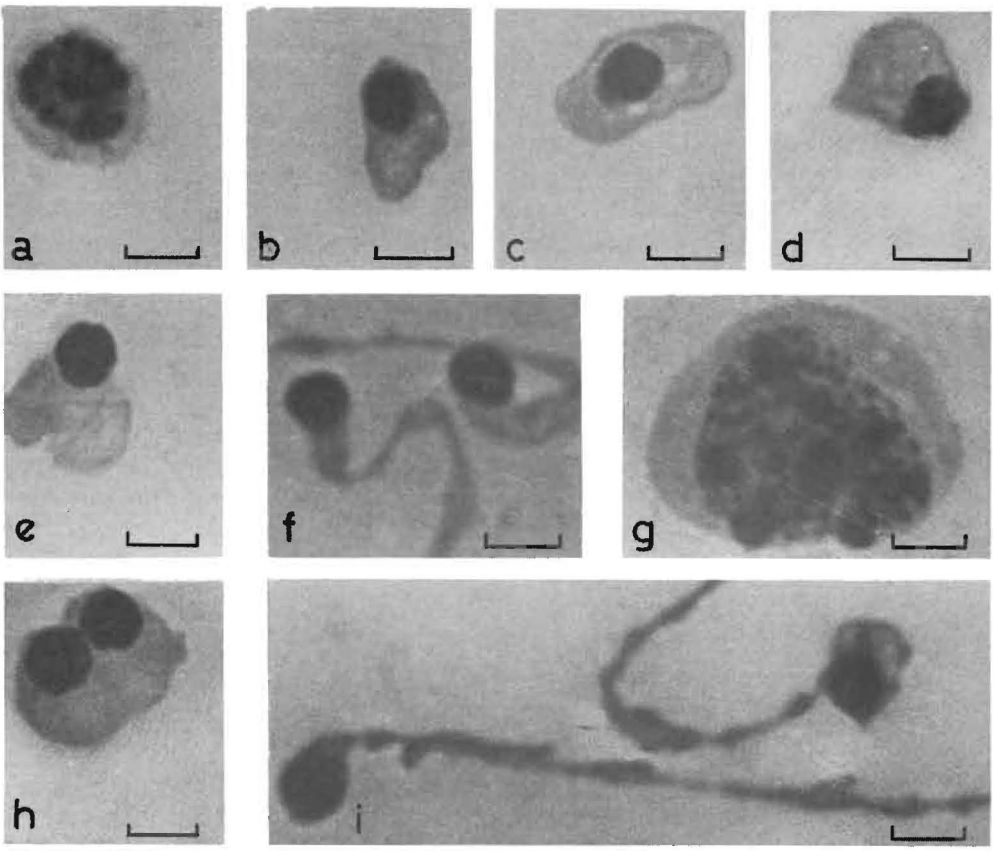

Fig. 4. A photomicrograph of representatives of different types of haemocytes in *D. aereus*; a, b—Prohaemocytes; c, d, i, j, k, l—Plasmatocytes; f—Granular haemocytes; e—Oenocytoids; m—Adipohaemocytes (arrow); g—Spherulocytes. (Scale bar = 10 μm.).

Regular cyclic responses to ecdysis were noticed unanimously in all the species, but their population decreases after third ecdysis (Figs. 5c, 6c, 7c, and 8c).

Spherulocytes

These haemocytes are round or oval in shape, and measure 8 to 18 μm × 10 to 28 μm., and accumulate large spherules in their cytoplasm. The nucleus appears as a cluster of chromatin balls (Figs. li, j; 2f; 3h and 4g). The spherulocytes (SPs) in *D. maculatus* are not clearly identified in Giemsa stained preparations. But under phase contrast microscope they are found to contain colourless spherules which sometimes looked like additional nuclei.

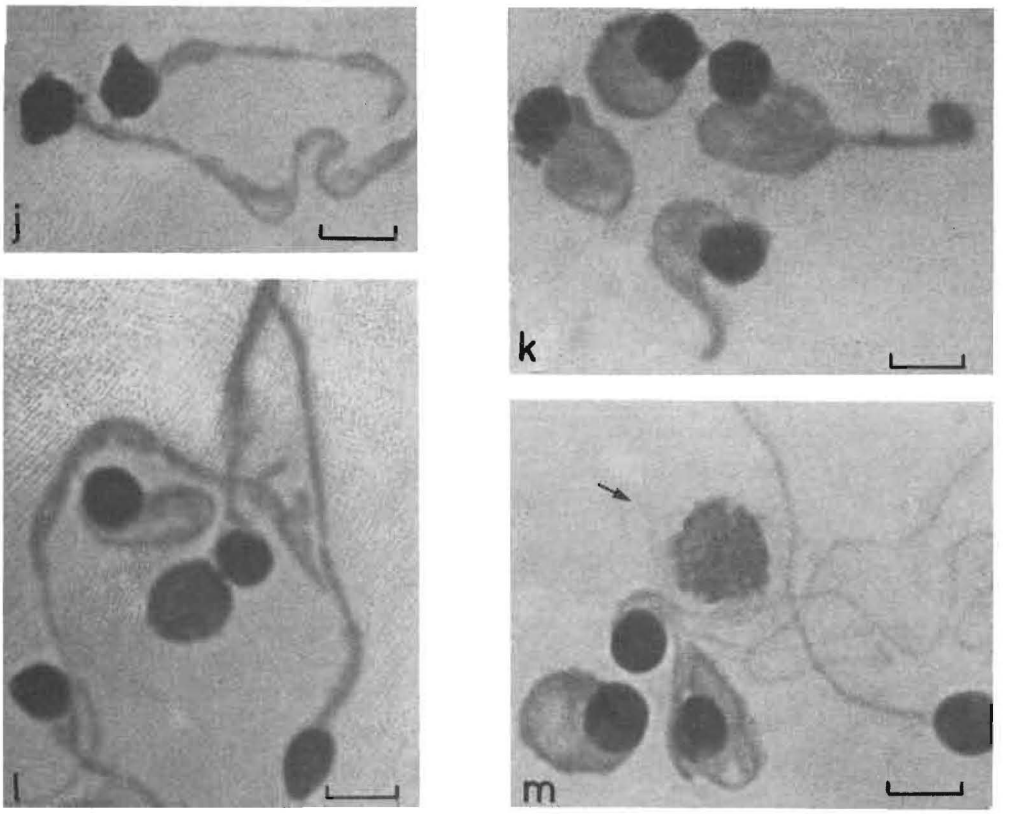

Fig. 4 (cont.)

The SPs are not found in the larval and adult stages of *D. vulpinus* (Fig 6a). The other three species contained less than five percent of SPs, except in *D. aereus* where their percentage exceeded 10%. The largest SPs are reported from *D. aereus* and the smallest from *D. maculatus* (Figs. 5, 6, 7, and 8).

Oenocytoids

Oenocytoids (OEs) (Figs. 1g; 2g; 3g and 4e) are characterised by a thick and dark stained cytoplasm, eccentric nucleus, and large vacuoles in the cytoplasm. Under phase contrast microscope they appear to have some kind of fibrous material in the cytoplasm. They are either round or slightly oval, but the nucleus is more or

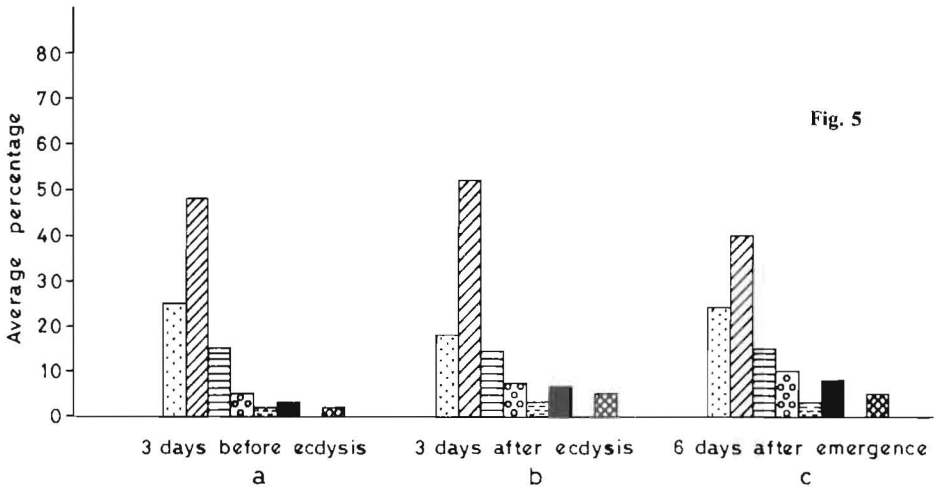

Fig. 5

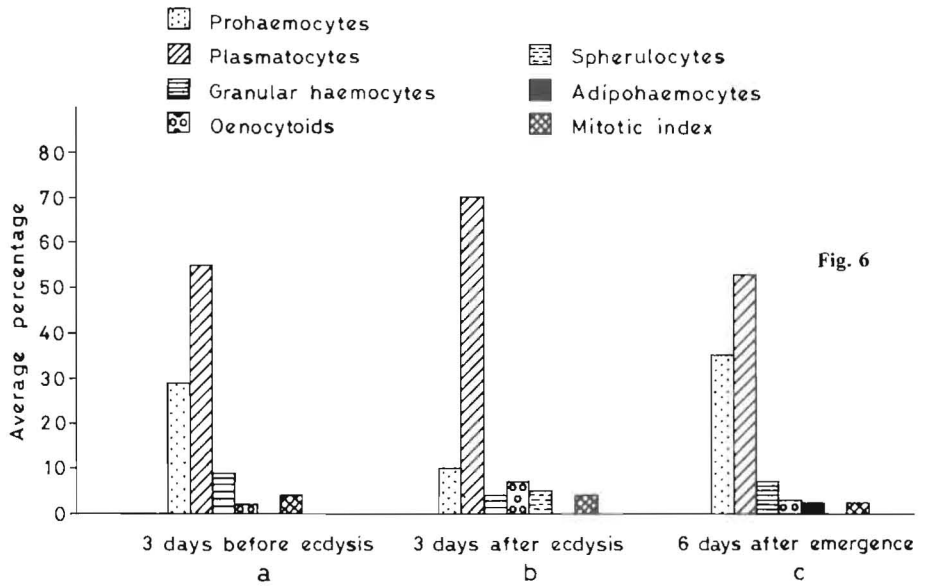

Fig. 6

Figs. 5 and 6. Histogram showing cyclic changes in the haemocyte population due to ecdysis in *D. maculatus* and *D. vulpinus* respectively.

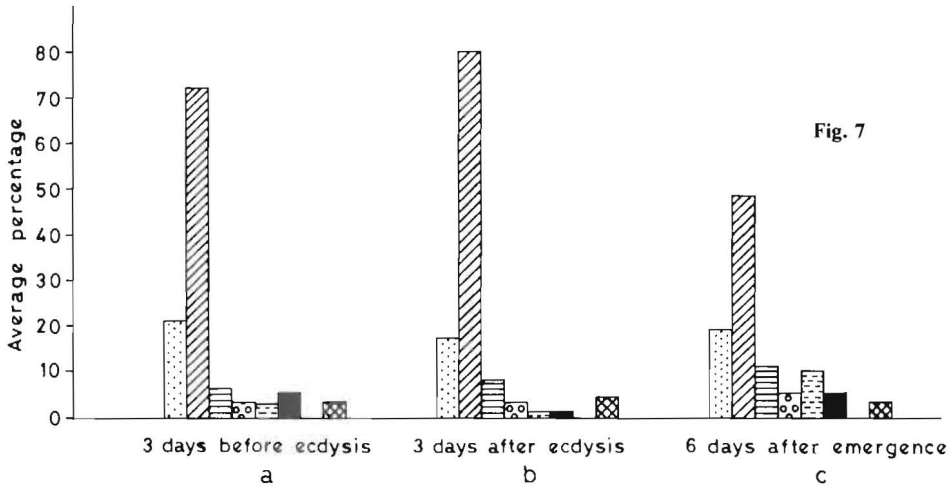

Fig. 7

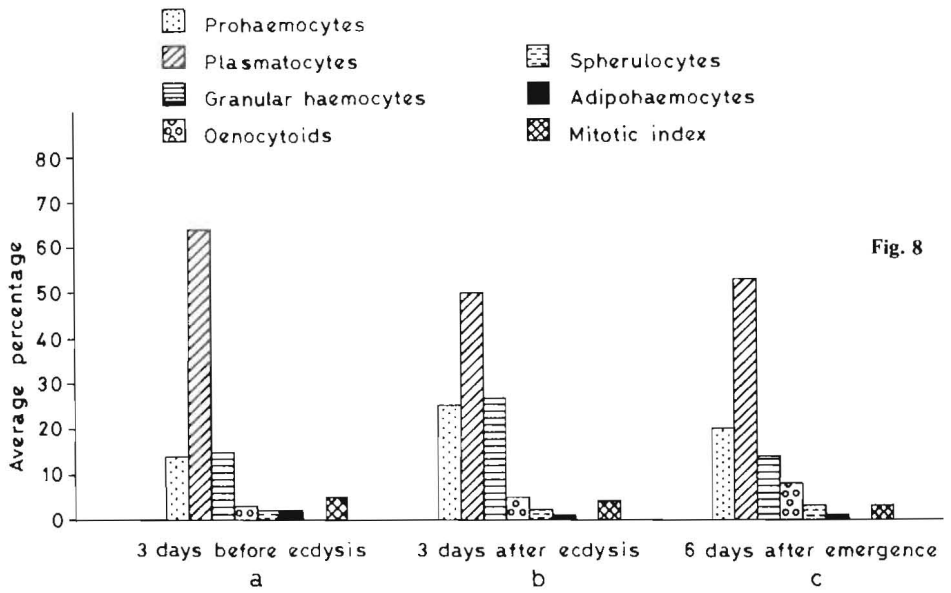

Fig. 8

Figs. 7 and 8. Histogram showing population variation in the different types of haemocytes: 7—*H. illigeri* and 8 in *D. aereus*.

less round or kidney shaped (Fig. 2j). They measure from 12 to 20 μm among all four beetles; the largest size being found in *D. vulpinus* and the smallest size in *D. aereus*. Their nuclei vary from 8 to 12 μm in width and 9 to 15 μm in length and their variation was really in accordance with the size of their whole cell and the ratio remained constant with little variation in all the four species.

Their population in all species studied remains under five percent, but their response to the changes due to ecdysis is similar to that of SPs (Figs. 5b, 6b, 7b and 8b), i.e. it decreases before ecdysis and increases after ecdysis.

Adipohaemocytes

Adipohaemocytes (ADs) are identified on the basis of accumulated lipid globules in their cytoplasm; they are mostly round in shape. Occasionally, these cells appear as a cluster of colourless globules in *D. maculatus* (Fig. 1h), *D. vulpinus* (Fig. 2g) *H. illigeri* (Figs. 3h, i, j, k) and *D. aereus* (Fig. 4m). The cells measure from 10–22 μm, in diameter, and each globule measures 0.5–1.5 μm.

The nucleus in these cells is not clear due to a covering of fat globules around it. It is round in shape and measures from 6–10 μm, highly basophilic and occasionally eccentric.

The ADs are the category which remain unavailable throughout the larval stages of *D. vulpinus*, whereas among adults of all four species, they constitute from two to five percent of the total population. The response to ecdysis is a decrease in their population up to three days after ecdysis. Afterwards, they regain the percentage mentioned (Figs. 5b, 6b, 7b and 8b).

Cyclic changes in differential haemocyte counts

A considerable change in the haemocyte population was the main feature noticed in all the species studied and this occurred more prominently in the respective percentages of the PRs and PLs. Among aquatic beetles the differential counts responded to an increase in the number of PLs and a decrease in the PRs and GRs (Figs. 5b, 6b, 7b and 8b) three days after third ecdysis.

Normally, the bulk of the population belonged to the PLs, while the PRs were second in number and the GRs third in percentage. Other categories remained in very few numbers, mostly less than five percent (Figs. 5. 6, 7 and 8) in the larval and adult stages of all the species studied.

Discussion

In view of the consensus evolved on the subject of naming the haemocytes in recent scientific research, the present investigation suggests the presence of a

maximum of six categories of haemocytes, (PRs, PLs, GRs, OEs, ADs, SPs), in *D. vulpinus.*; *D. maculatus*, *H. illigeri* and *D. aereus.* The former two species are terrestrial, whereas the latter two are aquatic so as to make the results more comparative.

The PRs, often called stem cells or proleucocytes, are the smallest haemocytes and have a comparatively large nucleus in respect to the size of their corresponding cells. Their essential occurrence throughout the life cycle of all the species determines their motherly role in the production of the other types of haemocytes. It also serves to confirm the acceptability of the idea of presenting the name PRs as more appropriate than leucocytes (Jones 1962; Wigglesworth 1965). These are definitely comparable to the leucocytes of vertebrate blood but perform few other functions like transformation into other types, unlike the leucocytes. Thus, the term PRs is more acceptable and justified. The use of other less popular terminology, accepted by only a few authors, is avoided in order to enhance the availability of comparable studies. The PRs are similar in character to the PRs that were reported by Jones (1962) in *Rhodnius prolixus*, and in *Dysdercus cingulatus* by Zaidi and Khan (1974).

The PLs, with a large cytoplasmic area, are polymorphic haemocytes acquiring different shapes in all four species studied. In *D. aereus*, over sixty percent of PLs have long threadlike extentions measuring up to 50 μm whereas, in *H. illigeri* the majority of the PLs are round or oval shaped. PLs are also named as phagocytes because of their phagocytic nature.

PLs are considered as acquired forms of PRs and it is sometimes, in the case of immature PLs in their intermediate stages, difficult to distinguish them from PRs. Results are available, which confirm the idea of the transformation of PLs into GRs (Gupta 1979).

The term GRs refers to the cells having cytoplasm with prominent granules; these were differently termed as coagulocytes (Devauchelle 1971), and as ADs by Francois (1975). In fact the GRs contain minute granules, whereas SPs include large spherules. The SPs are clearer under the phase contrast microscope than in stained preparations. The OEs, SPs, and ADs are described in accordance with the system adopted by Gupta (1979) and Jones (1962). Each of these types generally constituted less than 10% among all four species studied.

OEs are generally haemocytes with an eccentric nucleus and thick cytoplasm, with the occasional occurrence of one or more large vacuoles. The SPs are usually found having large spherules in the cytoplasm. These spherules sometimes make it difficult to distinguish the nucleus. The spherule cells are reported as annucleate cells by Kayya and Ratcliffe (1982) in some dipterous insects.

The ADs include lipid droplets within the cytoplasm. Raina and Bell (1976) found GRs with lipid droplets which he described as a transitory form of GRs, but which are really adipohaemocytes.

The present investigations support the postulations of Gupta (1979), considering PRs transitory to PLs and PLs to GRs and GRs as a transitional stage between OEs, SPs and ADs.

The consideration of ADs as GRs or the presence of lipid droplets in granular haemocytes by Raina and Bell (1976), is in agreement with the idea of acquiring different structures by PRs to form PLs than PLs to GRs and GRs to OEs, SPs and ADs. Jones (1967) observed changes in the haemocyte population of the different types due to ecdysis, an increase in the percentage of the plasmatocytes and a decrease in the granular haemocyte, following the ecdysis.

The present findings indicate that the haemocyte population changes in the fourth instar larvae of all four species studied due to ecdysis.

The PRs undergo a considerable reduction just after ecdysis that lasts for three days, coinciding with the increase in the percentage of the PLs, which denotes their transformation to the PLs. The number increased is not equal to the decrease in the PRs which also suggests the direct transformation of PLs into GRs.

Tauber (1937) reported changes in the mitotically dividing cells and a decrease prior to ecdysis and an increase after ecdysis. Mitotic index in two species, *D. vulpinus* and *H. illigeri* showed an increase after ecdysis. In the two other species, viz. *D. maculatus* and *D. aereus*, more changes in mitotic index were not observed.

The considerable changes in the percentage of PLs and PRs are important because of the role they play in response to the ecdysis in all four species studied. The mode of changes in the number of PLs is inversely proportional to the changes in the percentage of PRs. This inverse relationship may be interpreted as the transformation of PRs into PLs. Further, the inter-stages are occasionally available to conform this relationship.

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دراسة مقارنة لخلايا الدم في بعض انواع الخنافس

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قسم علم الحيوان ، كلية العلوم ، جامعة الملك سعود ، ص . ب : ٢٤٥٥ ،
الرياض : ١١٤٥١ ، المملكة العربية السعودية

لقد وجد بين انواع الخنافس الاربعة التي درست وهي *D. vulpinus* و *D. maculatus* و *H. illegeri* و *D. aereus* ان هناك ستة اشكال من خلايا الدم متميزة عن بعضها باستعمال المجهر الضوئي وهي :
خلايا الدم الاولية (Prohaemocytes) وخلايا الدم البلازمية (Plasmatocytes) وخلايا الدم الحبيبية (Granular haemocytes) وخلايا الاينوسيتويد (Oenocytoids) والخلايا الدموية الدهنية (Adipohaemocytes) وخلايا الدم الكروية (Spherule haemocytes) .
هذه الأشكال الستة ليست متوفرة في جميع أطوار كل حشرة ، فمثلا الخلايا الدموية الدهنية والكروية لم تظهر في الاطوار اليرقية من *D. maculatus* ولقد وصف تركيب ووجود هذه الخلايا الدموية بالاضافةالى التغيرات التركيبية التي تحدث لكل نوع منها ، ولوحظ أن وجود الاشكال المختلفة من خلايا الدم له علاقة بالاطوار المختلفة من دورة حياة الحشرة وكذلك حالتها الفسيولوجية .