Chapter III

# O B S E R V A T I O N S

# A N D

# R E S U L T S

Table 3.1 : Hemocyte types in various species

| Sr. No. | Order | S p e c i e s | Stage of development | PR | PL | GR | SP | CO | OE |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Thysanura | *Lepisma saccharina* | Adult | + | + | + | + | | + |
| 2 | Odonata | *Aeshna sp.* | Nymph | + | + | + | + | | + |
| 3 | Orthoptera | *Hieroglyphus nigrophlexus* | Adult | + | + | + | + | + | |
| 4 | | *Gryllus bimaculatus* | Adult | + | + | + | | + | |
| 5 | | *Gryllotalpa fossor* | Adult | + | + | + | | + | |
| 6 | Dictyoptera | *Periplaneta americana* | Adult | + | + | + | + | + | |
| 7 | Dermaptera | *Labidura riparia* | Adult | + | + | + | + | + | |
| 8 | Isoptera | *Microtermes sp.* | Adult (worker) | + | + | + | + | + | |
| 9 | Heteroptera | *Cimex rotundatus* | Adult | + | + | + | + | | + |
| 10 | | *Rhodnius prolixus* | Adult | + | + | + | | + | + |
| 11 | | *Notonecta sp.* | Adult | + | + | + | + | | + |
| 12 | | *Ranatra sordidula* | Adult | + | + | + | | | + |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 13 | Homoptera | Magicicada sp. | Adult | + | + | + | + | + | | |
| 14 | Neuroptera | Croce filipennis | Larva | + | + | + | + | | + | |
| 15 | Lepidoptera | Clania sp. | Larva | + | + | + | + | | + | |
| 16 | | Papilio demoleus | Larva | + | + | + | + | | + | |
| 17 | Diptera | Culex fatigans | Adult | + | + | + | + | | + | |
| 18 | | Musca (Musca) domestica | Adult | + | + | + | | | + | |
| 19 | | Lucilia sericata | Larva | + | + | + | + | + | + | |
| 20 | Coleoptera | Platynotus belli | Adult | + | + | + | + | + | | |
| 21 | | Heliocopris sp. | Larva | + | + | + | + | + | + | |
| 22 | | Zonabris pustulata | Adult | + | + | + | + | + | + | |
| 23 | | Hydaticus vittatus | Adult | + | + | + | + | + | + | |
| 24 | | Dineutus indicus | Adult | + | + | + | + | + | + | |
| 25 | Hymenoptera | Componotus compressus | Adult (Soldier) | + | + | + | + | | + | |
| 26 | | Apis mallifera | Adult (Worker) | + | + | + | + | + | | |
| 27 | | Chrysis ignita | Larva | + | + | + | + | + | + | |

**Results:**

Initially, unfixed and fixed wet blood films under light microscope and phase contrast optics were observed. All the hemocytes were refractile and vescicular. It was difficult to differentiate the "cell types". After waiting for 5-10 minutes six cell types were identified *in vitro*. By that time the cells attach to the substratum and spread out to reveal the intracellular details. *In vitro* studies of the unfixed wet films were particularly useful for identifying the coagulocytes and the amoeboid movement of the plasmatocytes.

Table 3.1 gives the information regarding the hemocyte types present in the insects studied presently. The total and differential count of the hemocytes in different species is presented in Table 3.2.

Fixed and stained preparations were used for the differential count of the hemocytes.

Of the thirteen orders examined, only three (Diptera: *L. sericata;* Coleoptera : *Heliocopris sp., Z. pustulata, H. vittatus, D. indicus;* and Hymenoptera : *C. ignita)* contained all the six types of hemocytes. They were prohemocytes, plasmatocytes, granulocytes, spherulocytes, coagulocytes and oenocytoids. The remaining ten orders

contained three basic types (prohemocytes, plasmatocytes and granulocytes) in addition to one or more of the other types.

The Apterygote order, Thysanura showed the presence of 5 types of hemocytes, namely, PRS, PLS, GRS, SPS and OES. This order was represented by Lepisma saccharina. From amongst the Hemimetabolous group, 7 orders were studied. These were Odonata, Orthoptera, Dictyoptera, Dermaptera, Isoptera, Heteroptera and Homoptera. Five cell types were recorded in odonata (PRS, PLS, GRS, SPS and OES). In H. nigrophlexus (Orthoptera) the cell types were PRS, PLS, GRS, SPS and COS. G. bimaculatus and G. fossor belonging to Orthoptera, however, possessed four cell types. These were PRS, PLS, GRS and COS. P. americana (Dictyoptera) had five cell types, namely PRS, PLS, GRS, SPS and COS. Dermaptera and Isoptera contained the same (as in the previous order) hemocyte types. Four species studied from Heteroptera showed variations. C. rotundatus had five types (PRS, PLS, GRS, SPS and OES) and R. prolixus possessed PRS, PLS, GRS, COS and OES. Notonecta showed the occurrence of PRS, PLS, GRS, SPS and OES; whereas R. sordidula had only 4 types (PRS, PLS, GRS and OES). Magicicada sp. (Homoptera) contained PRS, PLS, GRS, SPS and COS.

Five orders from Holometabola were studied (Neuroptera, Lepidoptera, Diptera, Coleoptera and Hymenoptera).

The larvae of C. filipennis (Neuroptera) contained PRS, PLS, GRS, SPS and OES. Two lepidopteran larvae studied possessed PRS, PLS, GRS, SPS and OES. M. domestica (Diptera) had four types of cells (PRS, PLS, GRS and OES). The adult of C. fatigans showed the presence of PRS, PLS, GRS, SPS and OES. In the larvae of L. sericata all the six types were observed. P. belli (Coleoptera) possessed PRS, PLS, GRS, SPS and COS. Other species, however, had all the six types of hemocytes. C. compressus (Hymenoptera) had PRS, PLS, GRS, SPS and OES. PRS, PLS, GRS, SPS and COS were present in A. mallifera. The larvae of C. ignita (Hymenoptera) had all the six types.

The details of the histomorphological peculiarities of the six types of hemocytes observed during the present investigation are given below :

Prohemocytes (PRS) :

Various characteristics of the PRS occurring in the insects examined are tabulated in Table 3.3.

PRS were found in all the insects studied presently. Their percentage, however, varied in different species. A minimum of 1% was present in insects like L. riparia and C. fatigans. In Z. pustulata they practically formed one fourth (24%) of the total hemocyte count. The percentage varied between 2 to 13 in the remaining insects.

## Abbreviations used in the Tables

1. A - Acidophilic
2. AG - Agranular
3. AM - Amoeboid
4. B - Basophilic
5. C - Central
6. CB - Compact in blocks
7. CO - Compact
8. E - Elliptical
9. EC - Eccentric
10. ED - Evenly distributed
11. EL - Elongate
12. F - Fibrillar
13. FG - Fine granular
14. FS - Few Spherules
15. G - Granular
16. H - Hyaline
17. HG - Homogenous
18. IB - Intense basophilic
19. K - Kidney shape
20. LG - Low granular
21. LSR - Large spherules
22. N - Neutrophilic
23. O - Oval
24. OB - Obscured
25. P - Polymorphic
26. PP - Polychromatophilic
27. PR - Peripheral
28. PY - Pyriform
29. R - Round
30. S - Spindle
31. SD - Spindle dominant
32. SP - Spherical
33. SSR - Small spherules
34. SR - Spherules
35. ST - Stellate
36. V - Variable
37. WA - Weak acidophilic
38. WB - Weak basophilic.

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## Captions to Figures

Prohemocytes of

Fig. 1 *P. americana* adult (x 675)

Fig. 2 *H. nigrophlexus* adult (x 300)

Fig. 3 *G. bimaculatus* adult; note nuclear chromatin (x 300)

Fig. 4 *Cicada sp.* adult (x 300)

Fig. 5 *R. prolixus* adult (x 675)

Fig. 6 *C. rotundatus* adult; note mitotic division (x 640)

Plasmatocytes of

Fig. 7 *R. prolixus*; transitional between PR and PL (x 675)

Fig. 8 *Aeshna sp*; note amoeboid form (x 675)

Fig. 9 *Heliocopris sp*; note amoeboid shape (x 600)

Fig. 10 *P. demoleus*; note spindle shape (x 600)

Fig. 11 *R. prolixus*; note binucleate condition (x 675)

Fig. 12 *L. saccharina*; Podocyte-like PL (x 675)

Fig. 13 *R. prolixus*; Vermicyte-like PL (x 675)

Fig. 14 *Clania sp.*; Sickle-shape PLS (X 600)

Fig. 15 *C. compressus*; Polymorphic PLS (x 640)

Fig. 16 *Cicada sp*; note very long tail-like process (x 300)

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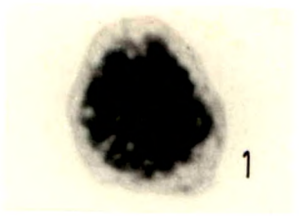
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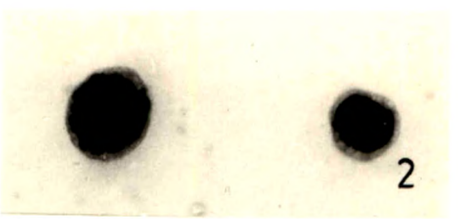
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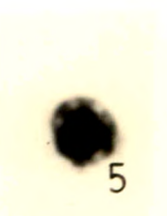
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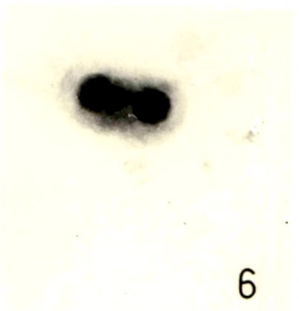
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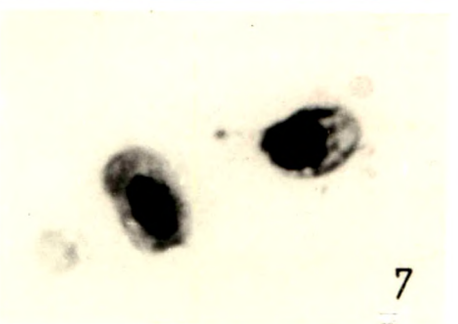
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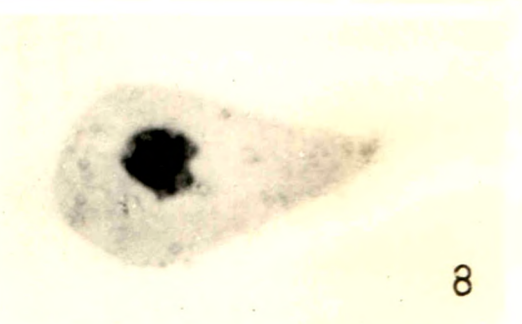
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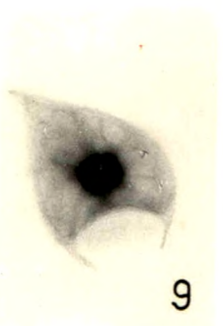
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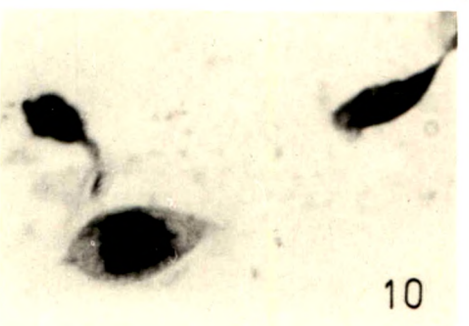
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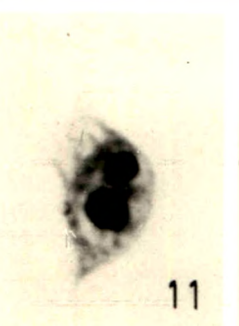
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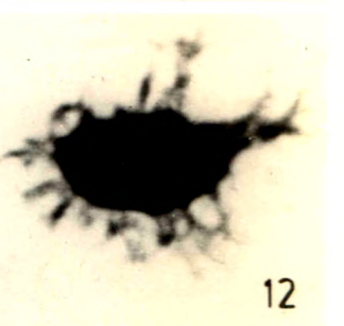
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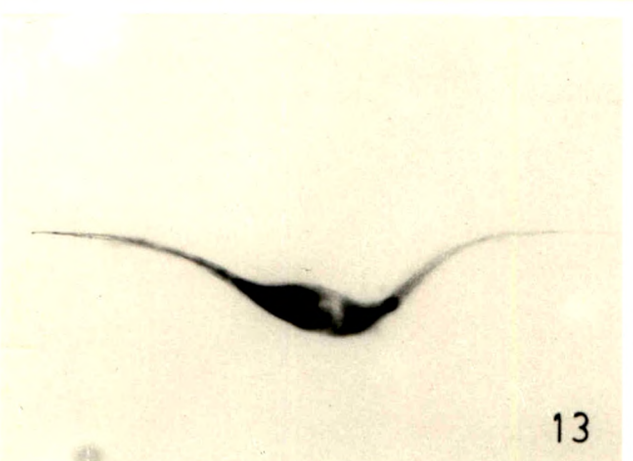
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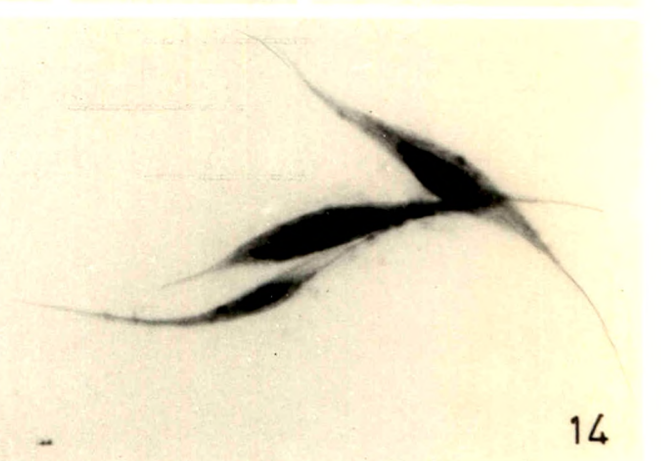
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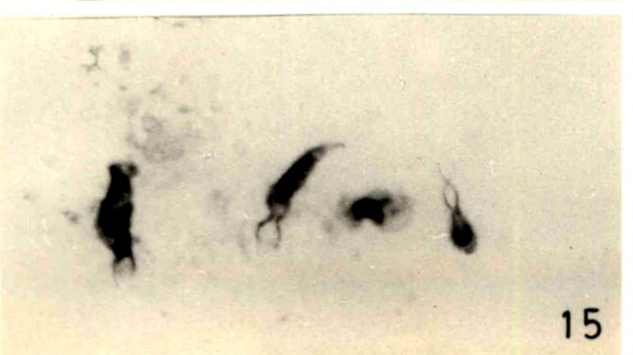
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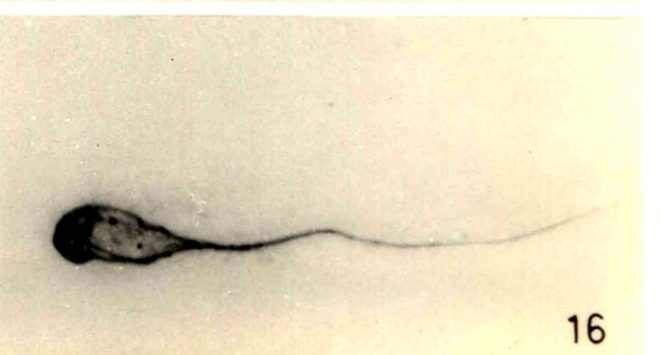
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The prohemocytes were round, oval or elliptical in outline (Fig.1-6). They were the most stable cells. Their diameter varied from 3 to 14 µm. In many of them the cytoplasm was dense in consistency. It was homogenous and basophilic. In certain insects the cytoplasm formed a thin rim like structure around the nucleus (Fig. 1 to 5). Granular or particulate inclusions were absent. The nuclei in PRS were centrally placed and compact in nature (Fig. 1,2,4,5). They were large in size. In many PRS the nuclei occupied approximately 70 to 90 % of the cell volume.

PRS were often very difficult to distinguish, particularly from the young plasmatocytes (PLS). Transitional forms between PRS and PLS were observed in many species. For the sake of convenience, the transitional forms (Fig.7) were regarded as PLS during differential count of hemocytes. The mitotic divisions were often seen among the PRS (Fig.6), indicating that the PRS are the main source of postembryonic hemocyte multiplication.

Plasmatocytes (PLS) :

Morphological peculiarities of the PLS are given in brief in Table 3.4.

From Table 3.2 it is evident that PLS are the main type of hemocytes in the hemolymph of many insect species. These cells were present in varying forms in all the insects

studied presently. It was difficult, hence, to distinguish them from the granulocytes (Fig.11). Amoeboid hemocytes of many species were very similar to PLS, except for the occurrence of many cytoplasmic granules.

PLS were small to large, polymorphic cells with variable size (Fig.7-16). They were round in C. ignita, L. riparia and few others. Their structure was amoeboid in Heliocopris sp. and R. sordidula (Fig.9), stellate in L. saccharina, sickle shaped in Clania sp. (Fig.14). In the remaining insects they were mostly spindle shaped (Fig.10).

The nuclei in them were round or oval or elongated. They were located centrally (Fig.8-16). The nuclear chromatin was distributed evenly. The cytoplasm was abundant in them and was basophilic. It was acidophilic in Aeshna and neutrophilic and acidophilic in Lepisma. The cytoplasm was agranular or finely granular or granular (Fig. 8 to 16). The nucleocytoplasmic ratio was low and ranged between 20 to 40%. In Rhodnius occasional binucleate cells were observed (Fig.11).

It was observed that the PLS were capable of attaching to the glass surface and spreading out to form cytoplasmic extensions. During this process the granules (when present) were retained in the endoplasm. Motile plasmatocytes were common in Orthoptera, Dictyoptera, Lepidoptera and Coleoptera.

## Captions to Figures

Granulocytes of

Fig. 17 P. americana; note refractile granules (x 300)
Fig. 18 H. nigroplexus;note central nucleus (x 640)
Fig. 19 R. prolixus; note neutrophilic granules (x 675)
Fig. 20 M. domestica; adult (x 675)
Fig. 21 H. vittatus adult; note finely granular cytoplasm (x 6

Spherulocytes of

Fig. 22 Notonecta sp; note polychromasia (x 675)
Fig. 23 C. fatigans; note central nucleus (x 675)
Fig. 24 H. nigroplexus; note obscuring of the nucleus (x 640)
Fig. 25 C. rotundatus adult (x 640)
Fig. 26 P. amesicana; typical small SPS (x 675)

Coagulocytes of

Fig. 27 M. domestica; note cart-wheel appearance (x 675)
Fig. 28 R. prolixus; note perinuclear cisterna (x 675)
Fig. 29 P. americana; note peripheral chromatin (x 675)
Fig. 30 L. sericata; Hyaline CO in the process of coagulation
Fig. 31 C. ignita; Hyaline COS producing rays (x 300)

Oenocytoids of

Fig. 32 Heliocopris sp.; note small size and eccentric nucleus (x 640)
Fig. 33 P. demoleus; note few neutrophilic spherules (x 640)
Fig. 34 L. saccharina adult (x 600)
Fig. 35 Aeshna sp; note eccentric nucleus
Fig. 36 C. rotundatus; note kidney shape eccentric nucleus (x 640)
Fig. 37 H. vittatus adult; note elongate shape (x 675)

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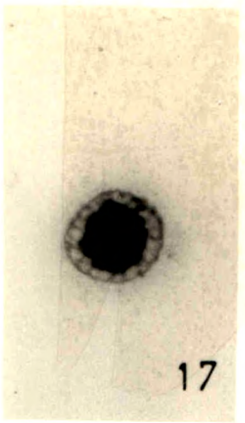
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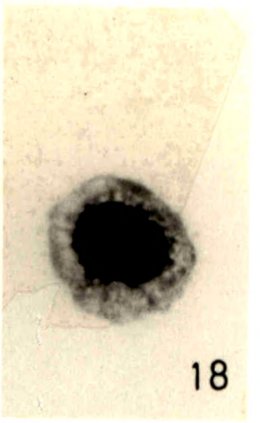
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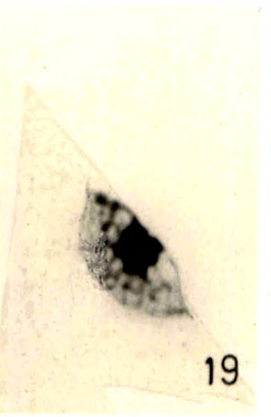
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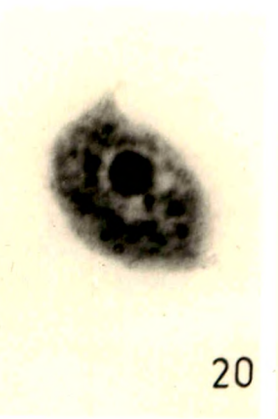
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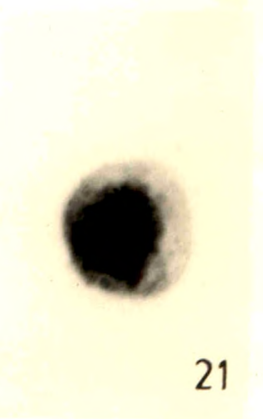
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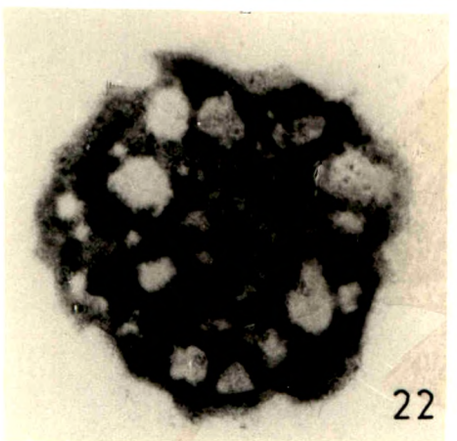
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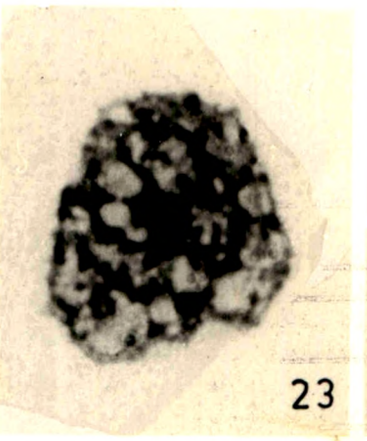
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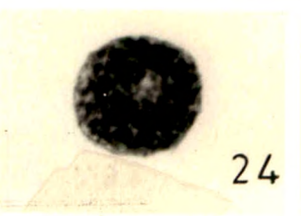
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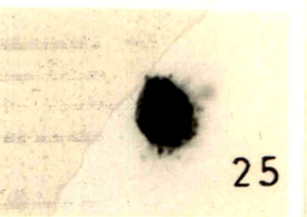
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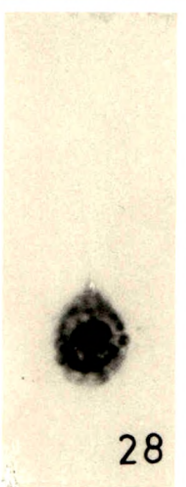
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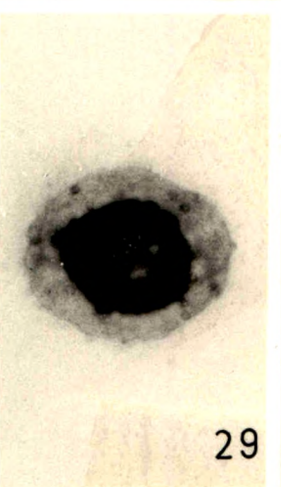
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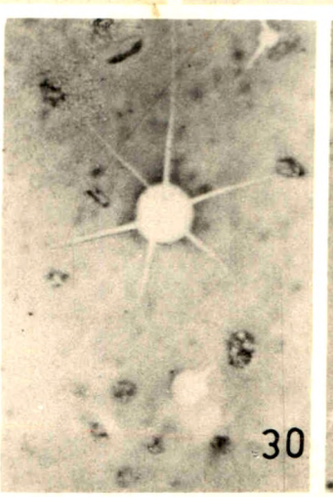
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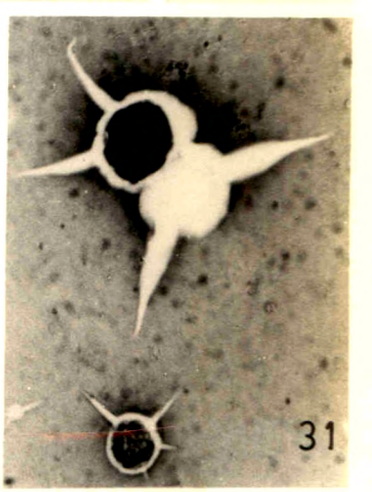
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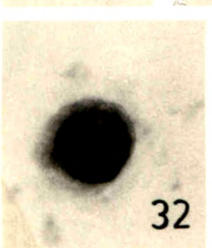
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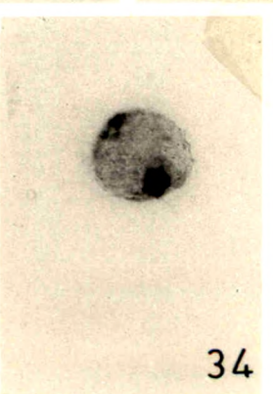
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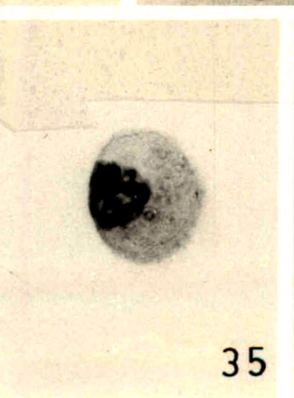
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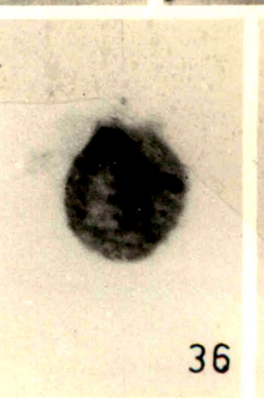
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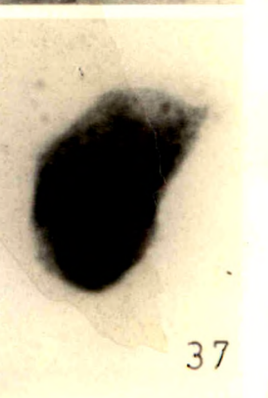
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Vacuolated PLS were common in the adults of Coleoptera. In the other orders, both the above types of PLS were found. Sickle shaped PLS were dominent in the larvae of Clania moth (Fig.14). They had about 2-4 µm width and about 16-20 µm length. More or less at the centre, there was a bulge, containing oval nucleus and agranular dense cytoplasm (Fig.14).

This elongated cells, with agranular or slightly granular cytoplasm, were observed in the odonata nymphs, adults of Hydaticus and Rhodnius (Fig.13). Large and extremely flat PLS like cell with several cytoplasmic extensions were found in Lepisma (Fig.12). In them the nuclei were large and centrally located.

Granulocytes (GRS) :

The insect species in which the GRS occur are given in Table 3.1. Their histomorphological characters are summerized in Table 3.5.

They were small in size in insects like Heliocopris sp. and L. sericata. In others they were large. Their shape varied from spherical to elongate (Fig. 17-21). They were oval in C. rotundatus, C. filipennis etc., spherical in L. riparia, G. fossor and round in Aeshna sp. and D. indicus. Typically they were pyriform in P. demoleus and elliptical in R. prolixus. Their diameter varied between 6 to 20 µm.

**Table 3.5 : Granulocyte Characteristics of Various Species**

| Sr. No. | Order | Species | Stage of development | Shape variation | | Size variation | | Nucleo-cytoplasmic ratio % | Position Nucleus | Nature of | | Staining reaction | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cell | Nucleus | Cell | Nucleus | | | Nuclear chromatin | Cytoplasmic inclusions | Cytoplasm | Nucleus |
| 1 | Thysanura | L. saccharina | Adult | R | O | 8 to 12 µm | 1 to 2 µm | 20 to 30 % | EC | ED | G | B/N | B |
| 2 | Odonata | Aeshna sp. | Nymph | R | O | 8 to 12 µm | 2 to 4 µm | 40 % | EC | ED | G | A/B | B |
| 3 | Orthoptera | H. nigrophlexus | Adult | R | O | 10 to 12 µm | 4 to 7 µm | 50 to 60 % | C | ED | G | A/N | B |
| 4 | | G. bimaculatus | Adult | R | O | 12 to 16 µm | 4 to 8 µm | 40 to 60 % | C | ED | G | B | B |
| 5 | | G. fossor | Adult | R/S | O | 10 to 20 µm<br>4 to 16 µm | 4 to 8 µm | 40 to 50 % | C/EC | ED | G | PP | B |
| 6 | Dictyoptera | P. americana | Adult | R/O | O | 10 to 15 µm | 6 to 10 µm | 60 to 70 % | C | ED | G | N/B | B |
| 7 | Dermaptera | L. riparia | Adult | R/S | O | 10 to 12 µm | 3 to 6 µm | 30 to 60 % | C | ED | G | B | B |
| 8 | Isoptera | Microtermes sp. | Adult (Worker) | R | O | 8 to 10 µm | 2 to 3 µm | 20 to 30 % | C | ED | G | B | B |
| 9 | Heteroptera | C. rotundatus | Adult | R/O | O | 10 to 12 µm | 4 to 6 µm | 40 to 60 % | C | ED | G | B | B |
| 10 | | R. prolixus | Adult | R/EL | O | 8 to 12 µm | 3 to 4 µm | 20 to 30 % | C/EC | ED | G | B/N | B |
| 11 | | Notonecta sp. | Adult | R | O | 8 to 16 µm | 4 to 8 µm | 30 to 50 % | C/EC | ED | G | B/A | B |
| 12 | | R. sordidula | Adult | R/O | O | 8 µm | 3 to 4 µm | 30 to 50 % | C | ED | G | B/N | B |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | Homoptera | Magicicada sp. | Adult | R | O | 12 to 20 µm | 4 to 8 µm | 30 to 40 % | C | ED | G | B/N | B |
| 14 | Neuroptera | C. filipennis | Larva | R/O | O | 10 to 12 µm | 3 to 5 µm | 30 to 40 % | C | ED | G | B/N | B |
| 15 | Lepidoptera | Clania sp. | Larva | R | R | 10 to 12 µm | 3 to 6 µm | 40 % | C | ED | G | B/N | B |
| 16 | | P. demoleus | Larva | R/PY | O | 10 to 12 µm | 4 to 6 µm | 40 to 50 % | C | ED | G | B/N | B |
| 17 | Diptera | C. fatigans | Adult | R | O | 10 to 12 µm | 4 to 6 µm | 40 to 60 % | C | ED | G | B/N | B |
| 18 | | M. domestica | Adult | R/O | O | 12 to 14 µm | 3 to 4 µm | 30 to 40 % | C | ED | G | B/N | B |
| 19 | | L. sericata | Larva | R/O | O | 8 to 10 µm | 4 to 6 µm | 50 to 60 % | C | ED | G | N/B | B |
| 20 | Coleoptera | P. belli | Adult | R/O | O | 10 µm | 3 to 6 µm | 40 to 60 % | C | ED | G | B | B |
| 21 | | Heliocopris sp. | Larva | R | O | 6 to 8 µm | 2 to 4 µm | 40 % | C | ED | G | B | B |
| 22 | | Z. pustulata | Adult | R | O | 8 to 10 µm | 2 to 4 µm | 30 to 40 % | C | ED | G | B | B |
| 23 | | H. vittatus | Adult | R/O | SP | 12 to 14 µm | 3 to 4 µm | 30 to 40 % | C | ED | G | B/N | B |
| 24 | | D. indicus | Adult | R | O | 8 to 10 µm | 3 to 4 µm | 40 % | C | ED | G | B/N | B |
| 25 | Hymenoptera | C. compressus | Adult (Soldier) | R | O | 8 to 12 µm | 3 to 4 µm | 30 to 40 % | C | ED | G | B/N | B |
| 26 | | A. mallifera | Adult (Worker) | R | O | 8 to 12 µm | 3 to 4 µm | 30 to 40 % | C/EC | ED | G | A | B |
| 27 | | C. ignita | Larva | R | O | 12 to 20 µm | 2 to 4 µm | 10 to 20 % | C/EC | ED | G | B | B |

The nucleus in most species was centrally located. In Thysanura and Odonata, however, it was eccentric. G. fossor, Notonecta sp., Rhodnius, A. mallifera and C. ignita were peculiar in the sense that, some of their GRS possessed central nuclei and others had eccentric nuclei (Fig.19). The chromatin was evenly distributed in the nuclei of all the insects examined. The nucleo-cytoplasmic ratio varied between 10-60 %. GRS contained basophilic cytoplasm with several granules varying in size (Fig. 17-21). The granules showed different staining properties. They were basophilic or neutrophilic or acidophilic. The granules in G. fossor were polychromatophilic. During in vitro studies, it was observed that the GRS release their granules in the hemolymph.

The GRS in R. prolixus (Fig. 19) were very conspicuous. In the remaining insects, there was some difficulty in distinguishing them from other hemocyte types (particularly the PLS). It was observed that, when the PLS count is low, GRS form the major composition of the hemolymph. It was also true vice-versa (Table 3.2). The GRS count was high in Thysanura, Isoptera, in one species each of Odonata, Heteroptera and Coleoptera, in two species of Diptera and three species of Hymenoptera.

Spherulocytes (SPS) :

The histomorphological details of SPS are illustrated

Table 3.6 : Spherulocyte Characteristics of various Species

| Sr. No. | Order | Species | Stage of development | Shape variation | | Size variation | | Nucleo-cytoplasmic ratio % | Position Nucleus | Nature of | | Staining reaction | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cell | Nucleus | Cell | Nucleus | | | Nuclear chromatin | Cytoplasmic inclusions | Cytoplasm | Nucleus |
| 1 | Thysanura | L. saccharina | Adult | R | O | 8 to 12 µm | 1 to 2 µm | 10 to 20 % | EC | ED | LSR | B/N | B |
| 2 | Odonata | Aeshna sp. | Nymph | R | O | 10 to 12 µm | 2 to 3 µm | 20 to 30 % | EC | ED | SR | A | B |
| 3 | Orthoptera | H. nigrophlexus | Adult | R | O | 16 to 20 µm | 4 to 6 µm | 20 to 30 % | EC/OB | ED | SR | A | B |
| 4 | | G. bimaculatus | Adult | | | | | | | | | | |
| 5 | | G. fosser | Adult | | | | | | | | | | |
| 6 | Dictyoptera | P. americana | Adult | R | O | 12 to 14 µm | 2 to 3 µm | 20 to 30 % | OB | ED | LSR | PP | B |
| 7 | Dermaptera | L. riparia | Adult | R/EL | O | 16 µm | 4 to 6 µm | 20 to 30 % | EC/C | ED | SR | B/N | B |
| 8 | Isoptera | Microtermes sp. | Adult (Worker) | R | O | 10 to 12 µm | 2 to 3 µm | 20 to 30 % | EC | ED | SR | B | B |
| 9 | Heteroptera | C. rotundatus | Adult | R | O/K | 10 to 16 µm | 3 to 4 µm | 20 to 30 % | EC | ED | LSR | A | B |
| 10 | | R. prolixus | Adult | | | | | | | | | | |
| 11 | | Notonecta sp. | Adult | R | O | 16 to 20 µm | 4 to 6 µm | 30 to 40 % | C/EC | ED | SR | PP | B |
| 12 | | R. sordidula | Adult | | | | | | | | | | |
| 13 | Homoptera | Magicicada sp. | Adult | R | O | 20 to 24 µm | 6 to 8 µm | 20 to 30 % | EC | ED | LSR | N | B |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | Neuroptera | C. filipennis | Larva | R | O | 12 to 16 µm | 4 to 6 µm | 30 to 40 % | C | ED | LSR | B | B |
| 15 | Lepidoptera | Clania sp. | Larva | R | O | 16 to 20 µm | 6 to 8 µm | 40 % | EC/OB | ED | LSR | N | B |
| 16 | | P. demoleus | Larva | R | O | 10 to 28 µm | 4 to 10 µm | 40 % | OB/BC | ED | SR | B/N | B |
| 17 | Diptera | C. fatigans | Adult | R | O | 10 to 12 µm | 2 to 3 µm | 20 to 30 % | C/EC | ED | LSR | A/N | B |
| 18 | | M. domestica | Adult | | | | | | | | | | |
| 19 | | L. sericata | Larva | R | O | 8 to 12 µm | 3 to 4 µm | 30 to 40 % | C | ED | LSR | B/N | B |
| 20 | Coleoptera | P. belli | Adult | R/O | O | 12 to 14 µm | 2 to 4 µm | 20 to 30 % | OB | ED | LSR | B/N | B |
| 21 | H | Heliocopris sp. | Larva | R | O | 12 µm | 4 to 6 µm | 30 to 40 % | C | ED | SSR | N | B |
| 22 | | Z. pustulata | Adult | R | O | 8 to 12 µm | 2 to 4 µm | 20 to 30 % | C | ED | SSR | B | N |
| 23 | | H. vittatus | Adult | R | O | 16 to 20 µm | 3 to 4 µm | 20 to 30 % | EC | C | LSR | B/N | B |
| 24 | | D. indicus | Adult | R | O | 12 to 16 µm | 1 to 3 µm | 10 to 30 % | C/EC | ED | LSR | PP | B |
| 25 | Hymenoptera | C. compressus | Adult (Soldier) | V | O | 8 to 10 µm | 2 to 3 µm | 20 to 30 % | OB | ED | LSR | A | B |
| 26 | | A. mallifera | Adult (Worker) | R | O | 12 to 16 µm | 2 to 3 µm | 20 to 30 % | EC | ED | SR | PP | B |
| 27 | | C. ignita | Larva | R | O | 20 to 22 µm | 2 to 3 µm | 10 to 20 % | OB | ED | LSR | B | B |

in Table.3.6.

Of the 27 species examined, SPS were present in all the insects except G. bimaculatus, G. fossor, R. prolixus, R. sordidula and M. domestica. They were round, oval and elongate cells (Fig. 22-26). Their diameter varied between 8 to 28 µm. SPS were usually larger than the GRS. They were filled with granules of larger size (spherules).

The nucleus in them was small and accounted for 10 to 40% of the cell volume. In some SPS, the nuclei were centrally placed (Fig. 23) and in others they were eccentric in position (Fig.25). The nuclei were obscured by the spherules in P. americana, P. belli and H. nigrophlexus (Fig.24). The chromatin was evenly distributed and was compact in nature. The cytoplasm in all the insects was basophilic and contained basophilic (Microtermes sp.), acidophilic (Aeshna sp., H. nigrophlexus, C. compressus), neutrophilic (Heliocopris sp., Clania sp.) or polychromatic (P. americana, Notonecta sp., D. indicus )spherules. The SPS were observed to release the spherules in the hemolymph by exocytosis (Fig.25).

SPS could be most easily identified in P. demoleus, H. nigrophlexus, C. rotundatus, L. sericata and C. ignita. Their identification was little difficult in some species of Coleoptera, Hymenoptera, Odonata and few others. The

SPS of C. compressus were usually of smaller size (approximately 8 µm) and those present in C. ignita were much larger (about 22 µm in diameter).

Coagulocytes (COS) :

Coagulocytes were present in Orthoptera, Dictyoptera, Dermaptera, Isoptera, Homoptera and Coleoptera. Their occurrence was prominent in R. prolixus, L. sericata, A. mallifera and C. ignita. Their distribution in insects is given in Table 3.1. Table 3.2 gives their count in different insects. The morphological peculiarities of them are shown in Table 3.7.

The COS were variously shaped (Figs. 27-31). They were round in G. bimaculatus, A. mallifera, C. ignita, stellate in Magicicada sp., oval in H. vittatus and pyriform in P. belli, H. vittatus. Their diameter varied between 8-16 µm. These cells were usually larger than the prohemocytes.

Their nuclei were round and usually occupied about 30-70 % of the cell volume. They were located centrally in G. fossor, Microtermes sp., Z. pustulata and P. americana (Fig.29). Their position was eccentric in Magicicada sp. and G. fossor. In many species well pronounced perinuclear cisternae were seen (Fig. 28). This feature helped in distinguishing these cells. Their cytoplasm was weakly basophilic in most of the cases. In H. nigrophlexus, however, it was

weakly acidophilic and neutrophilic in L. sericata and C. ignita.

The COS appeared hyaline in wet unfixed smears. Sooner or later they distintegrated and released their cytoplasmic contents into the hemolymph, initiating the process of coagulation. The COS, hence, were fragile and unstable cells.

In the larvae of C. ignita and L. sericata, the COS initially appeared as hyaline vescicles, showing cytoplasmic streaming movements. Soon cytoplasmic extensions radiated from them (Fig. 30, 31). These rays from many adjacent COS formed an intricate network of cytoplasmic threads. The hemolymph in their vicinity became thick embedding other cells in it. The latter process enhanced the release of their cytoplasmic content in the lymph. It was observed that the coagulocytes became active immediately within a minute or so, after bleeding. On the basis of these characters these cells could be distinguished from the others.

Oenocytoids (OES) :

OES were present in Thysanura, Odonata, Heteroptera, Neuroptera, Lepidoptera, Diptera, Coleoptera (except in P. belli) and Hymenoptera (except A. mallifera). Their occurrence in different species is given in Table 3.1. Table 3.2 gives their differential count. The histomorphological details of them are illustrated in Table 3.8.

The OES were relatively large sized hemocytes. Their diameter varied between 8-20 μm. These cells showed round or oval shape (Figs. 32-37). Round and oval combination occurred in M. domestica, L. saccharina, C. filipennis, D. indicus etc. They were oval in Clania sp., C. fatigans, P. demoleus and Z. pustulata.

In them the nuclei were small, compact and round or oval in outline (Figs. 32-37). The nuclei were eccentrically placed. The nucleocytoplasmic ratio varied between 20-40%. Their cytoplasm was usually homogenous and thick in consistancy. It was basophilic in all insects. There were many varieties of cytoplasmic contents. OES formed usually the minor cell type (Table.3.2). Exceptionally a large percentage of them was observed in C. rotundatus (about 25%).

In addition to the six types of hemocytes listed above, transitional forms between PRS ⟷ PLS, PLS ⟷ GRS, GRS ⟷ COS and GRS ⟷ SPS were distinguished in different insect species. Other hemolymph contents observed were lipomicrons, plastids, hemoconiae, microorganisms and helminth ova. The lipomicrons were small lipid droplets, presumably ejected from adipohemocytes. These are not classified into a type separately in the present investigation. They were filled with lipid droplets and had striking similarity with typical fat body cell. The plastids were non-nucleated inclusions. Hemoconiae occurred as fine

granules expelled from the lysing hemocytes.

Effect of Experimental Stress on Hemocyte Count :

A. Effect of Starvation :

Data in Table 3.9 indicates that the total hemocyte count (THC) of P. americana was much higher during starvation. Highest count was obtained on the 5th day (40,935 ± 1067). On 11th day, it was 32,031 ± 692. In comparison with the controls (22,291 ± 262), it was observed that the total count remained higher throughout the experiment (with some fluctuations).

After 2 days of starvation the count was 36,250 ± 883. During this period the PRS increased to 6% (control 3%). There was marginal decrease in the percentage of PLS and GRS. The number of SPS and COS did not alter. At the end of this period adipohemocytes appeared in the hemolymph and they constituted about 5% of the total count. On the third day of starvation an outbreak of the PLS was observed. Amongst them, the spindle forms were dominating. Adipohemocytes were not observed. On the fifth day there was reduction in PLS and increase in GRS number. At this time, even the PLS contained the granules. They were similar to the granulocytes in many respect. The GRS remained the dominating hemocyte type after 5th day of starvation. Increase in the number of COS was noticed from 6th day onwards. From 8th day onwards

distinct morphological changes were observed in some of the GRS. Their granules were pushed towards the periphery of the cell. The nuclear volume increased and chromatin loosened. The chromatin got dispersed. The perinuclear cisterna was observed. The above features are characteristics of the COS. Because of this reason, such cells were counted as COS. Hence, their number increased. Until the conclusion of the experiment (11th day), the GRS dominated the hemocyte population. They were followed by PLS, COS, SPS and PRS (in that order).

B. Effect of Dessication :

The fluctuations in THC during dessication are presented in Table 3.9.

During the first two days the count increased two fold. The count appeared to be normal on the 4th day. At the end of 5th day, however, there was an abrupt rise in the count (i.e. 91,000 $\pm$ 4,102). From the 6th day onwards there was a decrease in the count. This trend continued until 10th day. At that time the count was 8,875 $\pm$ 525.

After two days of dessication, the total count increased. On the 3rd day most of the PLS observed were either spindle shaped or had the appearance of vermicytes. On the 4th and 5th day, the plasmatocytes became polymorphic. DHC remained unchanged upto 8th day. From 8th day onwards the degenerative changes commenced in all the cell types.

These changes were more pronounced in GRS. The frequency was also more. Some granulocytes resembled the COS. From 10th day onwards the process of cell fragmentation was more frequent. The changes were so rapid that it was impossible to distinguish the cell types and hence, the THC and DHC were not done.

C. Combined Effect of Starvation and Dessication :

Table 3.9 illustrates the combined effect of starvation and dessication.

The total count increased significantly. Maximum count was obtained on the 4th day (88,687 ± 514). The count was minimum (44,687 ± 1067) on 10th day. The data obtained indicated that, although the counts were much higher than the control; there were fluctuations of great magnitude.

After 2 days, the GRS were the predominant type, the GRS and the PLS comprised the main bulk on the 4th day (about 86 to 89 %). The PRS increased to about 7-10 %, the COS about 3% and the SPS less than 1%. The increase in the COS was evident on the 5th day (about 6%). At the end of 7th day, however, the COS number dropped down below average (i.e. less than 3%). By the end of 8th day, cell degenarative process was initiated in the many PLS and GRS. In them the cell membrane was inconsistant. They were illdefined and lysed at several places. From 10th day onwards, the degenara-

tive process was enhanced. On the 11th day of starvation and dessication, surprisingly, some newly formed PLS and GRS were seen along with the old degenerating ones.

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