# ULTRASTRUCTURAL STUDIES OF THE HEMOCYTES OF PANSTRONGYLUS MEGISTUS (HEMIPTERA: REDUVIDAE)

#### MARGHERITA A. BARRACCO & CLARICE T. LOCH

Departamento de Biologia, Universidade Federal de Santa Catarina, Caixa Postal 476, 88049 Florianópolis, SC, Brasil

Ultrastructural analyses revealed the presence of six hemocyte types in the hemolymph of Panstrongylus megistus, partially confirming our previous results obtained through light microscopy. Prohemocytes: small, round hemocytes with a thin cytoplasm layer, especially rich in free ribosomes and poor in membranous systems. Plasmatocytes: polymorphic cells, whose cytoplasm contains many lysosomes and a well developed rough endoplasmic reticulum (RER). They are extremely phagocytic. Sometimes, they show a large vacuolation. Granulocytes: granular hemocytes whose granules show different degrees of electrondensity. Most of them, have an internal structuration. Coagulocytes: oval or elongated hemocytes, which show pronounced perinuclear cisternae as normally observed in coagulocytes. The cytoplasm is usually electrondense, poor in membranous systems and contains many labile granules. Oenocytoids: large and very stable hemocytes, whose homogeneous cytoplasm is rich in loose ribosomes and poor in membranous systems. Adipohemocytes: large cells, containing several characteristic lipid droplets. The cytoplasm is also rich in glycogen, RER and large mitochondria.

The total and differential hemocyte count (THC and DHC) were also calculated for this reduviid. THC increases from 2,900 hemocytes/mm³ of hemolymph in the 4th instar to 4,350 in the 5th and then, decreases to 1,950 in the adults. Plasmatocytes and coagulocytes are the predominant hemocyte types.

Key words: hemocytes - Panstrongylus megistus - Hemiptera - ultrastructure

Relatively few studies exist on the hemocytes of hemipterans as opposed to those concerning other insect orders, such as lepidoptera and coleoptera. The main ultrastructural observations on hemipteran hemocytes were carried out on the reduviid Rhodnius prolixus by Lai-Fook (1968; 1970). Dorn (1979), identified the hemocytes of the heteropteran Oncopeltus fasciatus, using the classification adopted by Lai-Fook. The hemocytes of R. prolixus were also deeply studied through light microscopy by Wigglesworth (1933; 1955) and Jones (1965). In spite of a large amount of information on the hemocytes of R. prolixus, their classification is still controversial.

The reduviid Panstrongylus megistus is, in Brazil, one of the most important vectors of the protozoan Trypanosoma cruzi which causes Chagas' disease. Their hemocytes have been previously described by us (Barracco et al.,

Supported by CNPq.

Received November 28, 1988. Accepted February 27, 1989.

1987) through light microscopy. In the present paper, we intend to complete our previous results through an ultrastructural study. These observations could be useful for a comparison with the hemocytes of R. prolixus and could lead to a more uniform nomenclature of blood cells in reduviids and possibly in other hemipterans.

Quantitative (THC) and qualitative (DHC) analyses on hemocyte population during the insect development, were also performed.

#### MATERIALS AND METHODS

All hemocyte analyses were performed on sylvatic 4th and 5th instar nymphs and adults of Panstrongylus megistus from the Island of Santa Catarina, non contaminated by the Trypanosoma cruzi. These animals were reared and maintained in our laboratory under controlled temperature (25-28 °C) and humidity (60-80%) and were fed with chicken blood, once every two weeks. The life cycle of P. megistus of the Island of Santa Catarina is circa

one year in our laboratory. The 4th instar period lasts 85 days and the 5th instar nymphs take about a 100 days for the last moult and to reach the adult stage.

Before collecting the hemolymph, the insects were placed on ice for 5-10 min for immobilization. Hemolymph was always obtained from a severed leg or antenna.

Electron microscopy - Hemolymph was directly collected into the fixative which consisted in a 2.5% glutaraldehyde solution in 0.2M phosphate buffer, pH = 7.2. Hemolymph was fixed for 1-2 h at 4 °C. Pellets of hemocytes obtained by low centrifugation, were washed three times in the same phosphate buffer. They were, then, postfixed in a 1% osmium tetroxyde solution, phosphate buffered, for 30 min at 4 °C and washed three times in a 0.2M NaCl solution. Hemocyte pellets were, then, contrasted with 1% uranyl acetate for 15-18 h at 4 °C and washed again in the same NaCl solution. After conventional dehydration in graded ethanol solutions, the blood cells were embedded in Spurr resin. Ultra-thin sections cut on LKB ultra-microtome were mounted on copper grids, contrasted with lead citrate and examined in a Zeiss-Em, operating at 60 KV.

Total hemocyte count (THC) per mm<sup>3</sup> of hemolymph — Hemolymph was diluted with a saline solution (Tauber & Yeager, 1935) and THC was determined using a Neubauer chamber and a procedure similar to that used for blood white cell counts.

Differential hemocyte count (DHC) — DHC was carried out by counting the different hemocyte types and calculating their relative percentages in Giemsa smears or by phase contrast observations of fresh blood smears.

### RESULTS

Six hemocyte types were identified in the hemolymph of sylvatic *P. megistus* through transmission electron microscopy (TEM) analyses:

Prohemocytes (Fig. 1): these are small cells (8-11  $\mu$ m), round or oval in shape. The large nucleus (6-9  $\mu$ m) fills the cell almost entirely and occasionally, contains nucleoli (Fig. 1-A). The thin cytoplasm layer is characterized by a

low concentration of intracellular organelles and is especially rich in free ribosomes. Golgi bodies and mitochondria may be found, and sometimes a poor developed rough endoplasmic reticulum (RER) is also observed. Microtubules are occasionally present.

Sometimes, prohemocytes can be taken for plasmatocytes, since transitional forms between them are frequently observed (Fig. 2-A and B).

TABLE I

Total hemocyte counts (THC) per mm<sup>3</sup> of hemolymph during the development of Panstrongylus megistus

	Stage of development						
Sample -	4th instar*	5th instar*	Adults**				
1	1,188	1,913	1,025				
2	1,700	2,213	1,063				
3	2,400	2,450	1,487				
4	2,463	2,563	1,625				
5	2,687	4,100	1,850				
6	2,713	4,337	2,025				
7	3,048	5,275	2,187				
8	3,812	6,000	2,362				
· 9	4,300	6,229	2,787				
10	4,588	8,287	2,937				
Mean	2,889.9	4,336.7	1,934.8				
SD	1,082.9	2,107.6	656.3				

<sup>\*</sup> Each sample includes the hemolymph of 4-5 nymphs.

Plasmatocytes (Figs 3-5): polymorphic hemocytes, larger than prohemocytes (10-28  $\mu$ m). The plasma membrane may have filopodia and vesicular or pinocytic invaginations. The nucleus (6-15  $\mu$ m), centrally located, shows scattered chromatin masses and sometimes nucleoli. The cytoplasm generally contains a well developed RER and several dark granules which could be lysosomes. In fact, plasmatocytes are extremely reactive to the Gomori substrate for acid phosphatase and are very active in phagocytosis (Barracco et al., 1987). They can be seen engulfing large amount of different particles, such as bacteria (Fig. 5-A and B), erythrocytes and others (Barracco et al., 1987). Mitochondria, Golgi bodies, scattered microtubules and a large amount of loose ribosomes are also present.

<sup>\*\*</sup> Each sample includes the hemolymph of 9-12 adults (males and females).

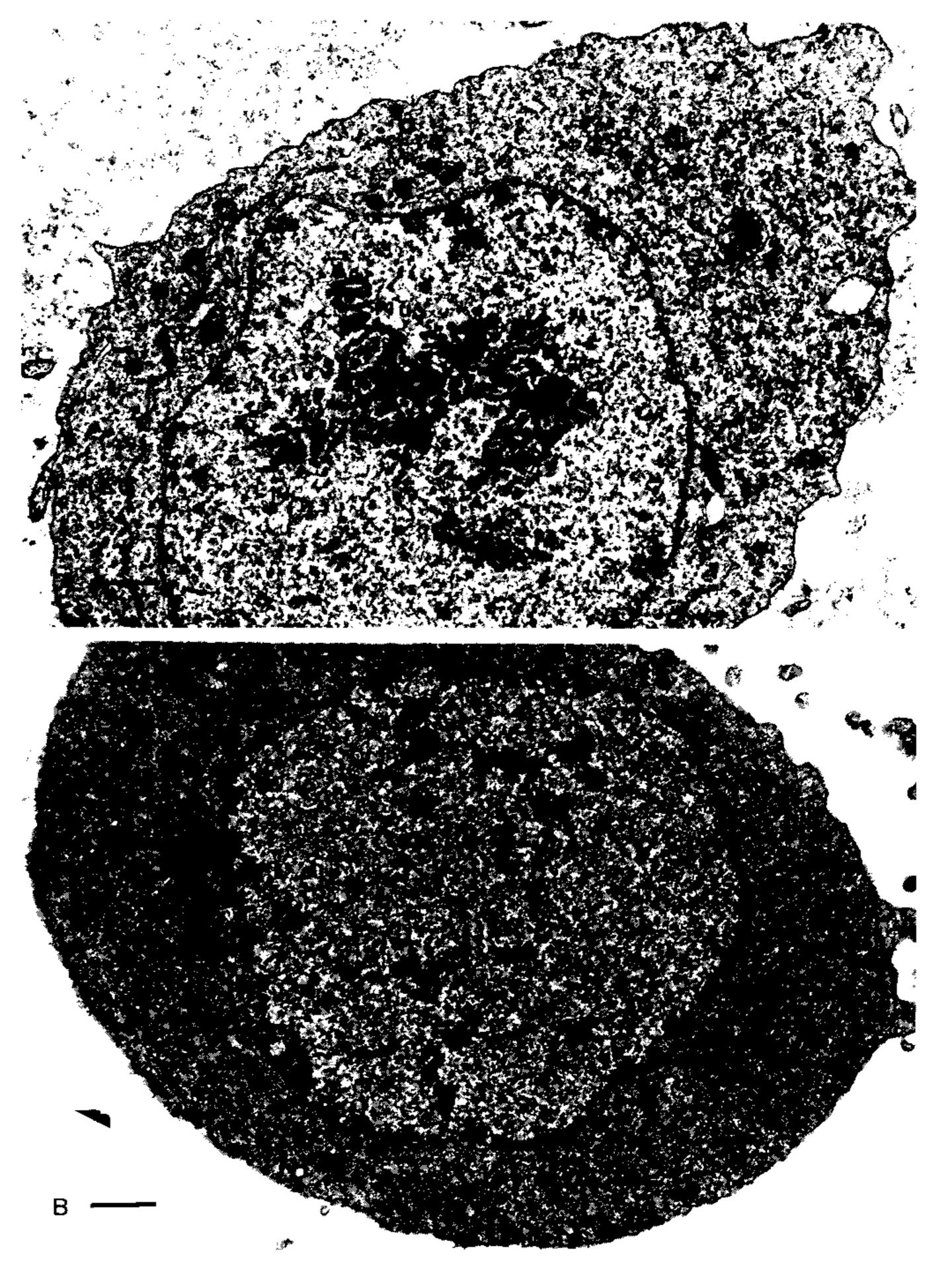


Fig. 1-A and B: prohemocytes. Mi = mitochondria; N = nucleus; Nu = nucleulus; REG = granular endoplasmic reticulum; Ri = ribosomes. Bar = 1  $\mu$ m.

Sometimes, the cytoplasm shows a large vacuolation (Fig. 4-A) and seems to be invaded by a meshwork of channels originated from the invagination of the plasma membrane.

It can be seen in Table II, that plasmato-

cytes, together with granulocytes, are the most abundant hemocytes.

Granulocytes (Figs 6 and 7): spherical, oval or elongated hemocytes (10-28  $\mu$ m). The plasma membrane may have filopodia and vesicular

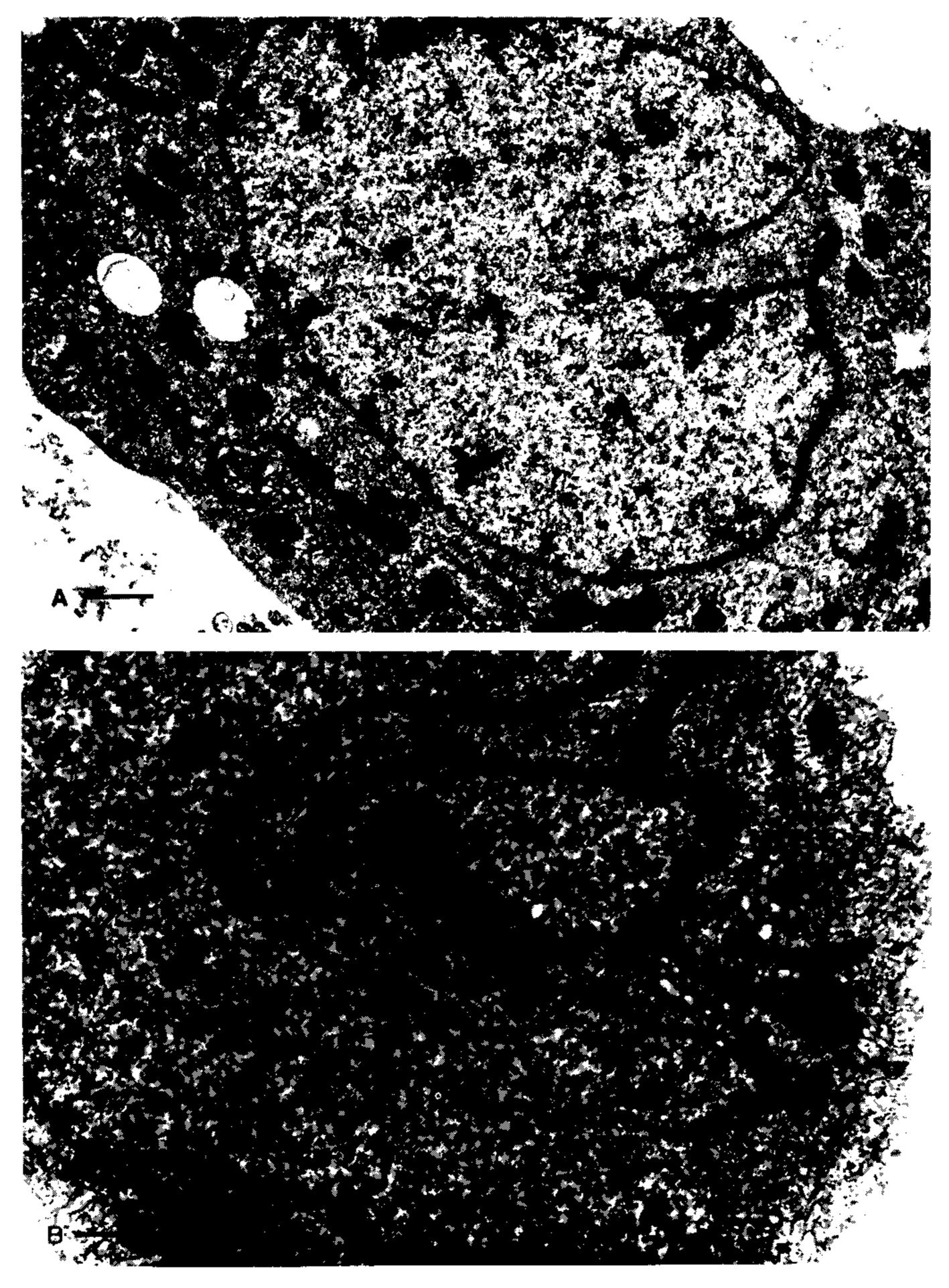


Fig. 2-A and B: transitional forms between prohemocytes and plasmatocytes. Go = Golgi complex; Mi = mito-chondria; Mt = microtubules; N = nucleus; REG = granular endoplasmic reticulum; Ri = ribosomes. Bar = 1  $\mu$ m.

invaginations. The nucleus  $(6-15 \mu m)$  is, in general, centrally located and may contain scattered chromatin masses and occasionally nucleoli. The cytoplasm is characteristically granular. The granules are of different sizes and

can be structured (Fig. 7-B) or apparently structureless, showing different degrees of electrondensity (Fig. 7-A). Supposedly, the most electrondense granules must represent a final stage of maturation and some of them are

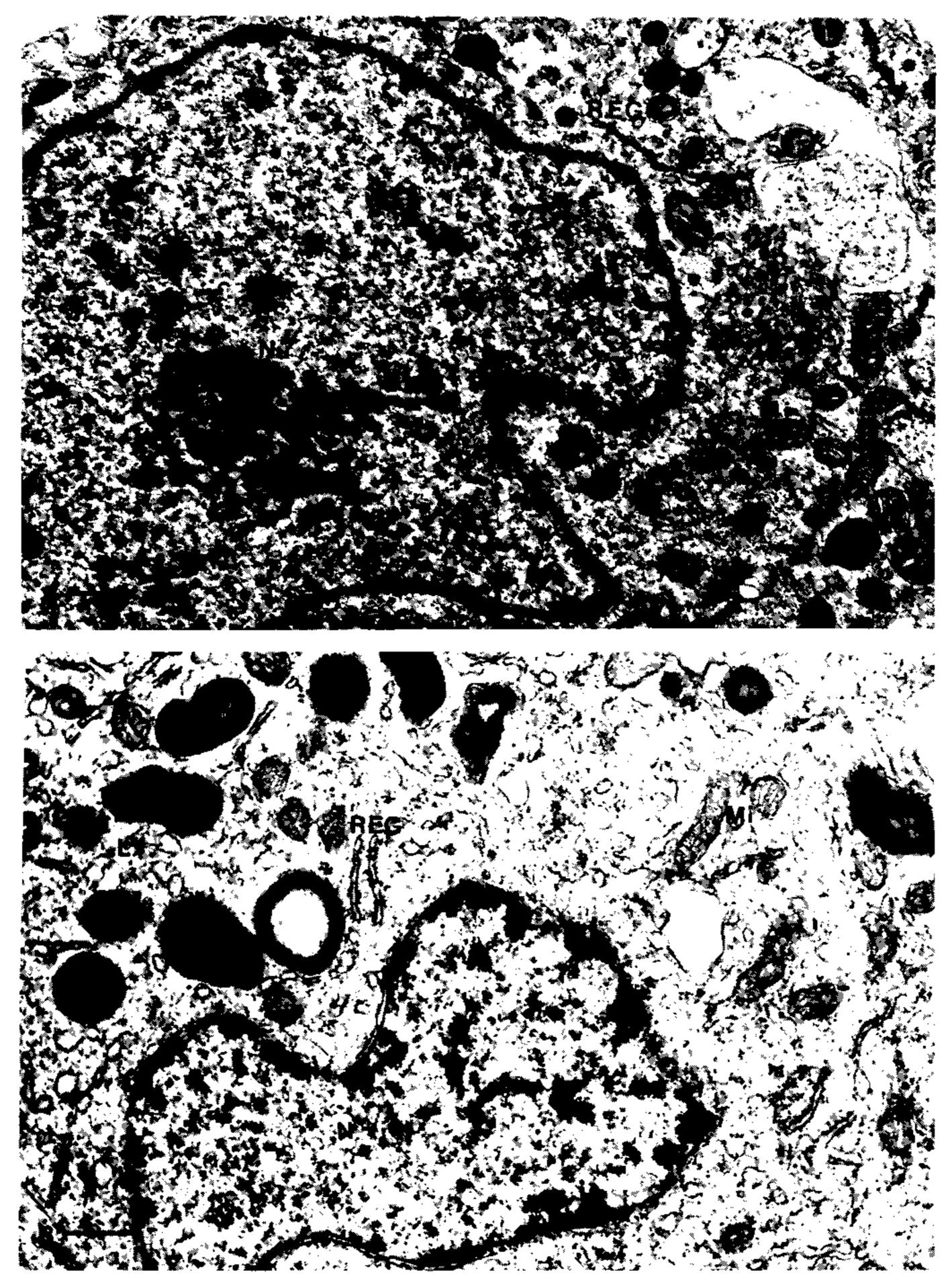


Fig. 3-A and B: plasmatocytes. Ly = lysosomes; Mi = mitochondria; N = nucleus; Nu = nucleulus; REG = granular endoplasmic reticulum; Ri = ribosomes. Bar = 1  $\mu$ m.

probably released into the hemolymph, since 7-B), as already described for other insects. they are seen very near to cell periphery (Fig. 6-A). The structured granules seem to contain an arrangement of micro-microtubules (Fig.

Histochemically, they proved to contain polysaccharides, since they are strongly positive to periodic-acid-Schiff (PAS). They are

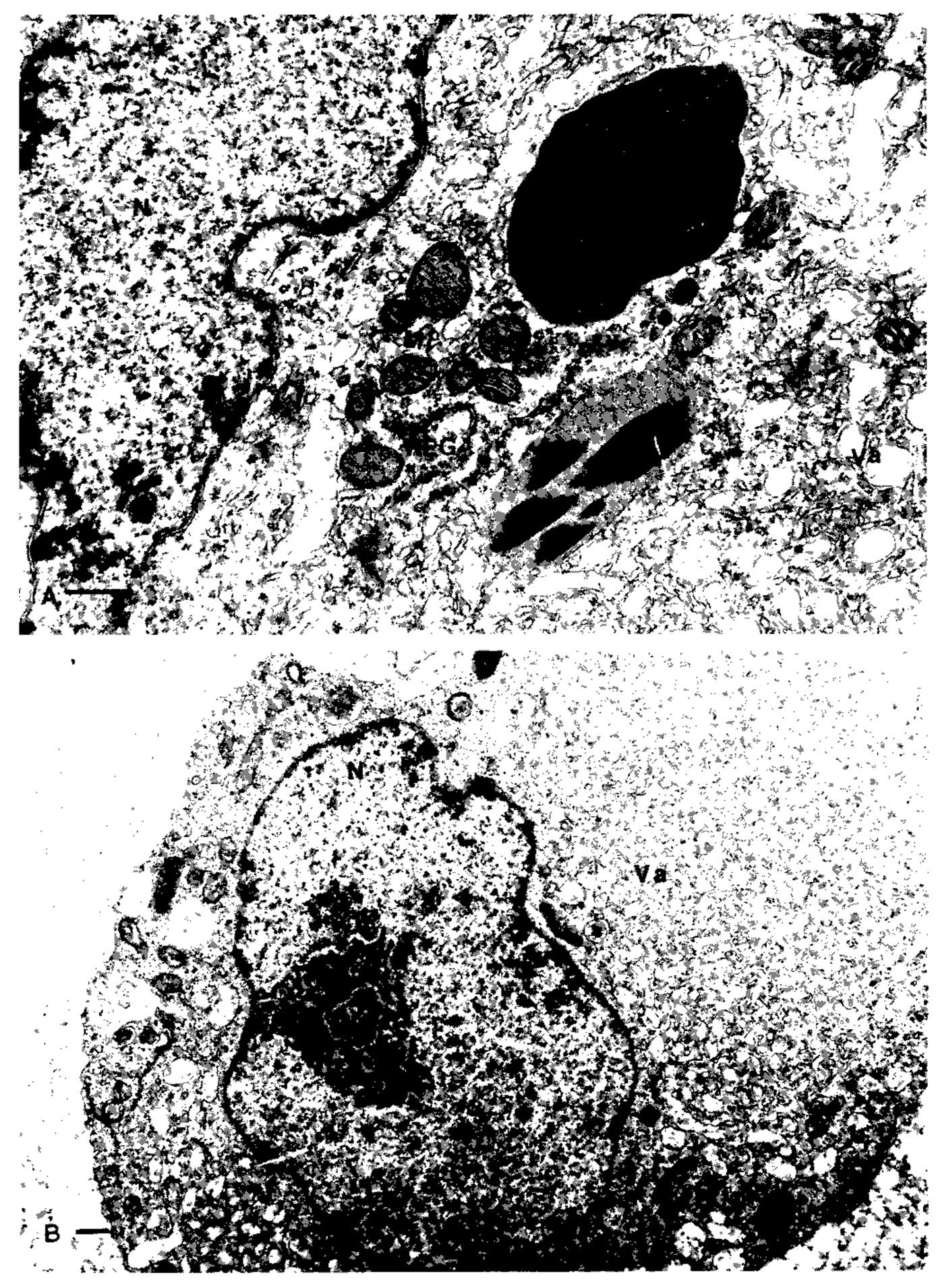


Fig. 4-A and B: plasmatocytes showing vacuolated cytoplasm. Ly = lysosomes; Mi = mitochondria; N = nucleus; Nu = nucleulus; REG = granular endoplasmic reticulum; Va = vacuolation. Bar = 1  $\mu$ m.

especially rich in sulformucins and sulfated glycosaminoglicans, since they are stained by Alcian Blue pH = 1.0 (Barracco et al., 1987).

In addition to granules, the cytoplasm is also

rich in RER which shows different degrees of cisternae dilatation (Fig. 6-B). The cisternae content could be the precursor of granules. The cytoplasm also has mitochondria, Golgi bodies and a large amount of free ribosomes.

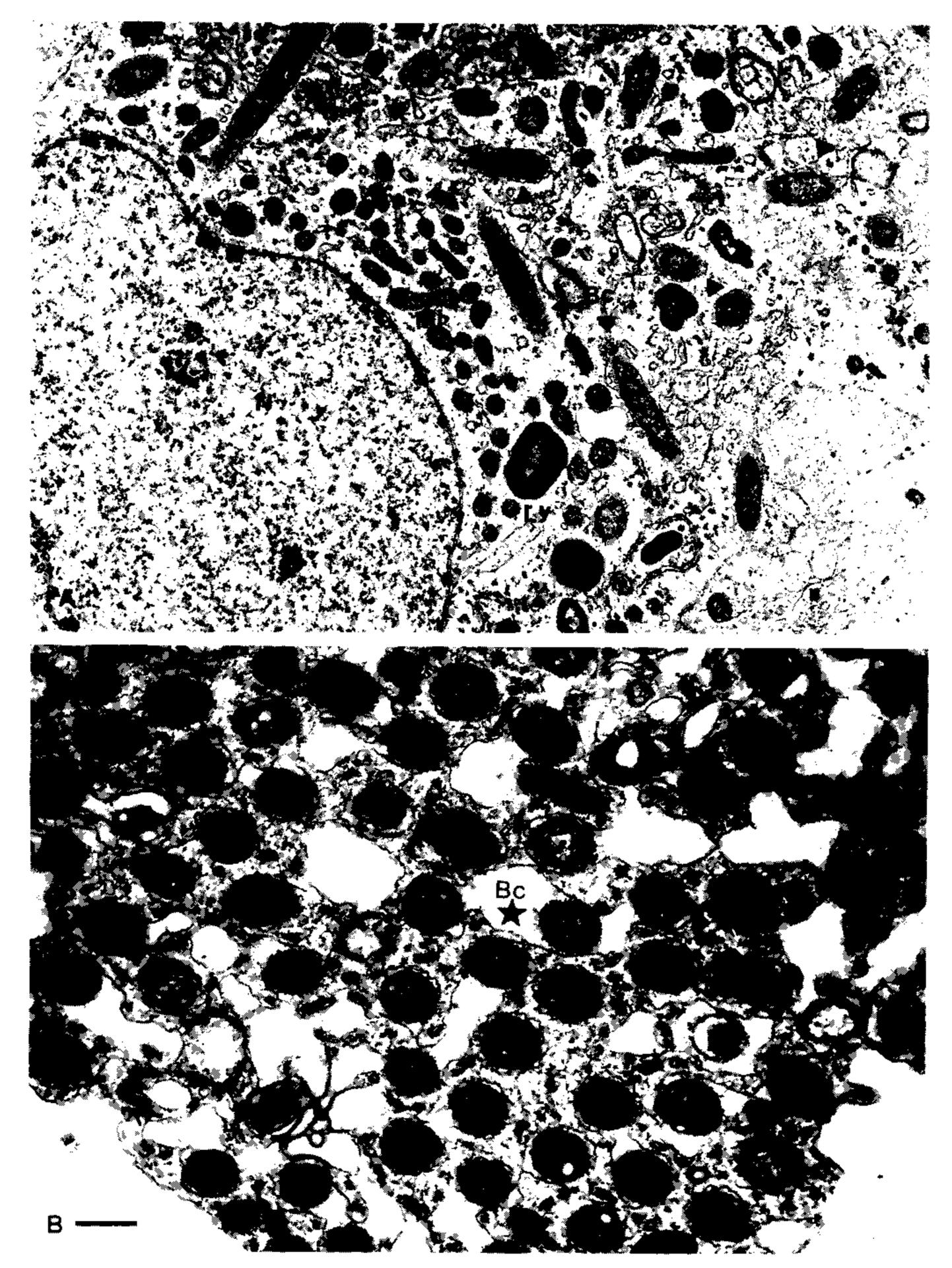


Fig. 5-A and B: phagocytosis of bacteria by plasmatocytes. Bc\* = bacteria in all directions. Ly = lysosomes;  $Mi = mitochondria; N = nucleus. Bar = 1 \mu m.$ 

(9-21  $\mu$ m), spherical, oval or elongated hemocytes. The plasma membrane generally does not have filopodia or vesicular invaginations. The nucleus is relatively small (5-9  $\mu$ m) and some-

Coagulocytes (Figs 8 and 9): small to large times eccentrical. It usually presents characteristic chromatin masses, especially when observed in Giemsa smears. Barracco et al. (1987) classified them, as oenocytoids, but TEM analyses revealed that they are ultra-

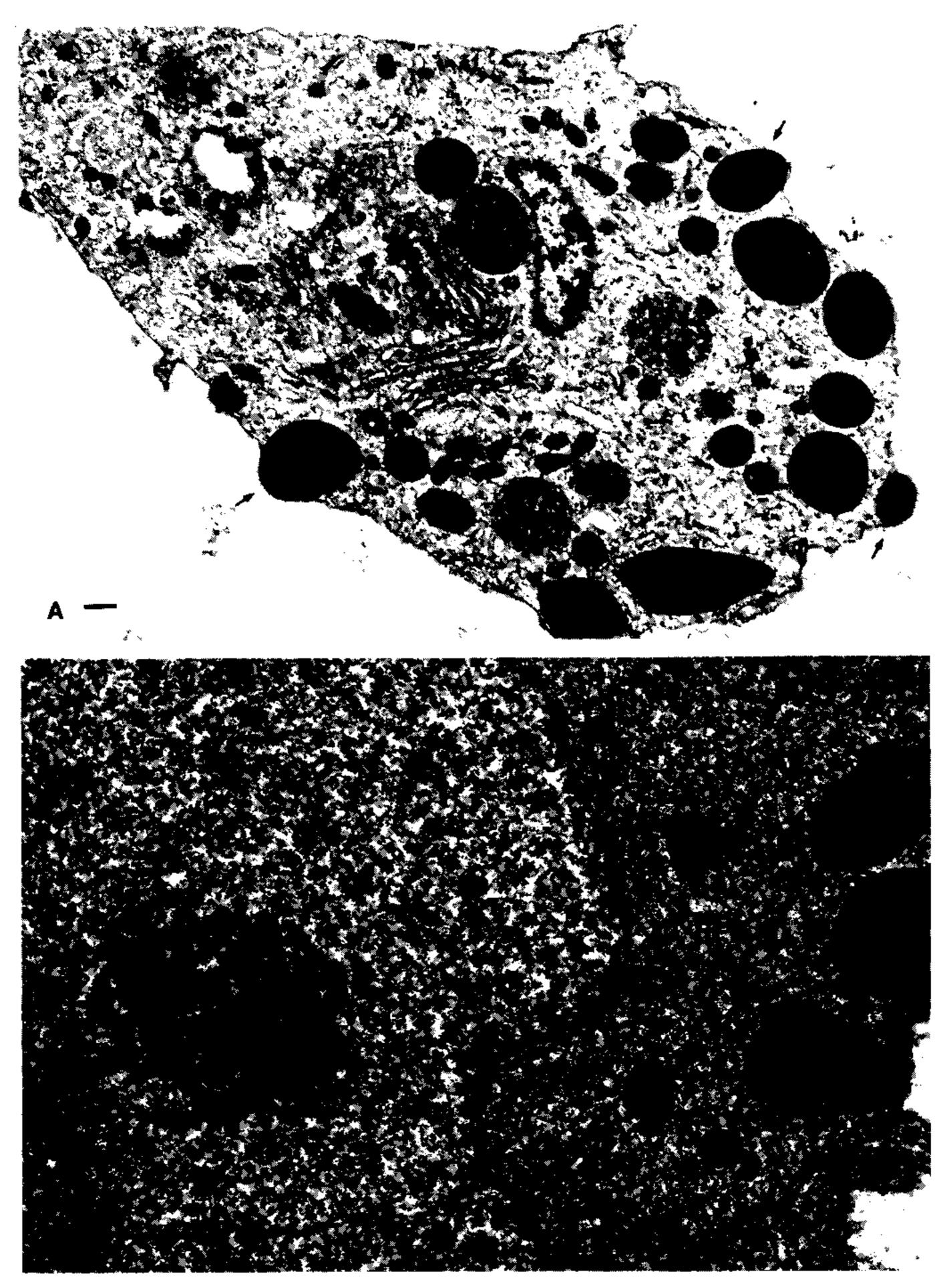


Fig. 6-A and B: granulocytes. In A, the arrows indicate the probable extrusion of granules. In B, we can observe the dilatation of REG disternae containing a low electrondense material. Gr = granules; N = nucleus; Nu = nucleulus; REG = granular endoplasmic reticulum. Bar = 1  $\mu$ m.

They have very characteristic perinuclear cisternae (Fig. 9-A and B), as usually described fixation and staining methods. In Giemsa for coagulocytes. The cytoplasm is dense and homogeneous and frequently electrondense.

structurally more similar to coagulocytes. It contains many granules which seem to be very labile, since they are easily removed after smears, these granules are lost and give rise to small round vacuoles (Barracco et al., 1987).

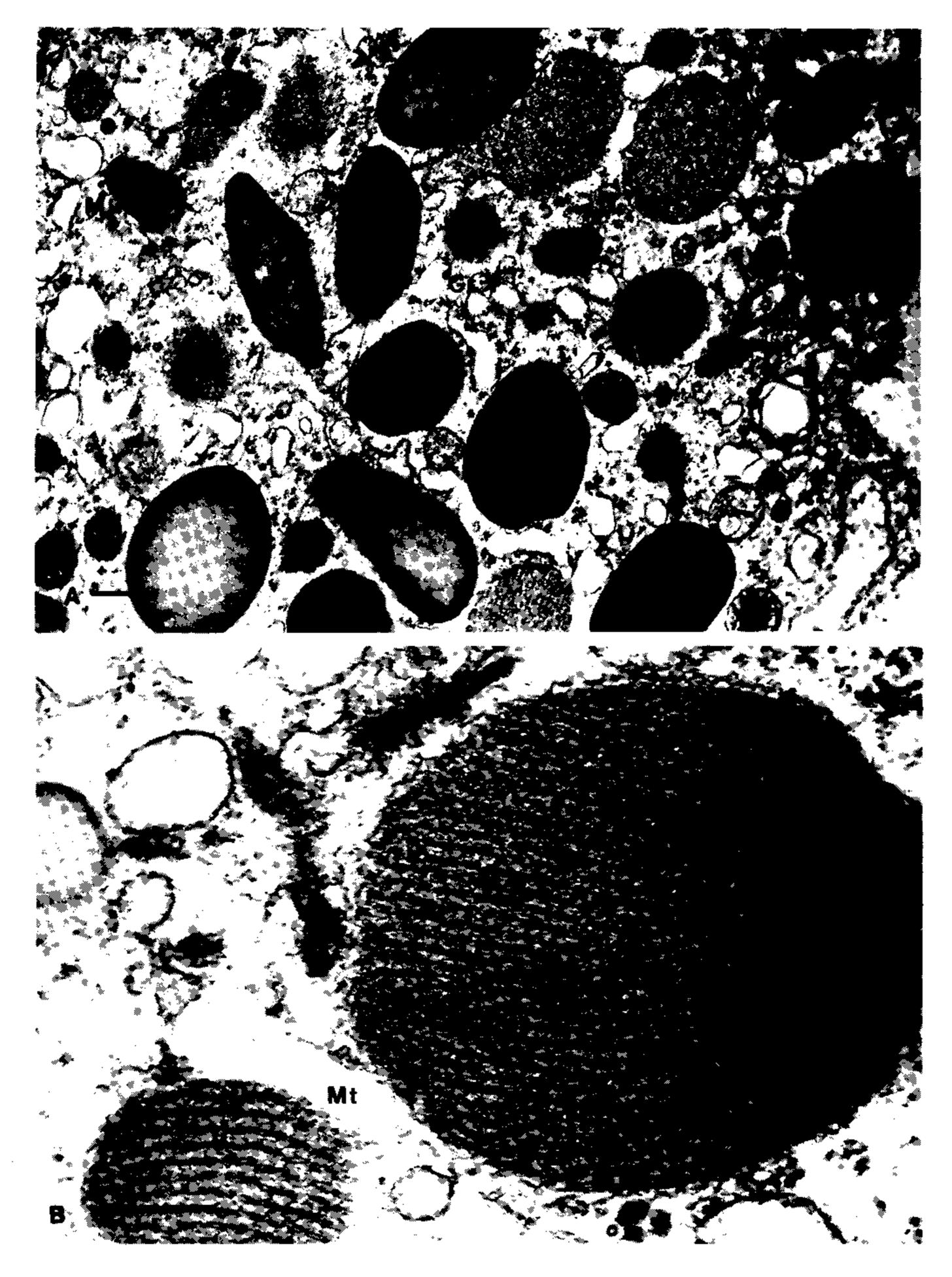


Fig. 7-A and B: granulocytes. In A, the granules (Gr) show different degrees of electrondensity. Some of them are structured. In B, detail of a structured granule (Gs) which seems to contain micro-microtubules. Gr\* = granules in all directions; Mi = mitochondria; Mt = microtubule. Bar = 1  $\mu$ m.

These granules are often explosively discharged in the hemolymph, when coagulocytes are observed *in vitro*. However, islets of coagulation

are not formed around them as it usually occurs with other insects. The hemolymph of *P. megistus* does not clot even after a long period of air

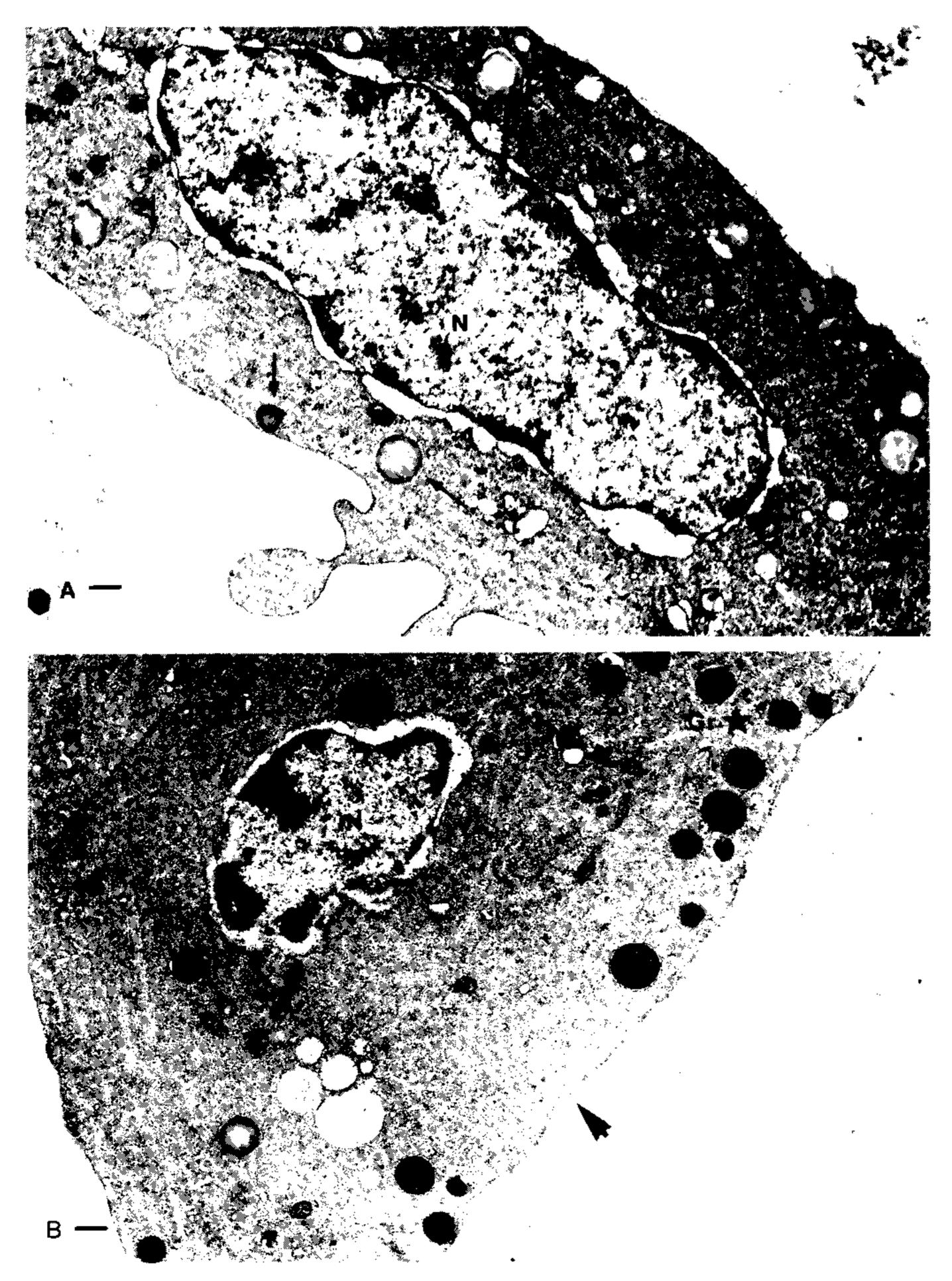


Fig. 8-A and B: coagulocytes showing swollen perinuclear disternae. In B, the arrowhead shows a membrane microrupture as usually described in coagulocytes. Arrows and Gr\* indicate labile granules in all directions. N = nucleus. Bar = 1  $\mu$ m.

exposition. The cytoplasm of coagulocytes is very poor in membranous organelles. Scattered microtubules are usually present (Fig. 9-A).

Oenocytoids (Fig. 10): round or oval, large

hemocytes (18-34  $\mu$ m), very stable in their morphology. Their cytoplasm is also dense and homogenous and very basophilic in Giemsa stainings. In TEM analyses they are usually much less electrondense than coagulocytes and

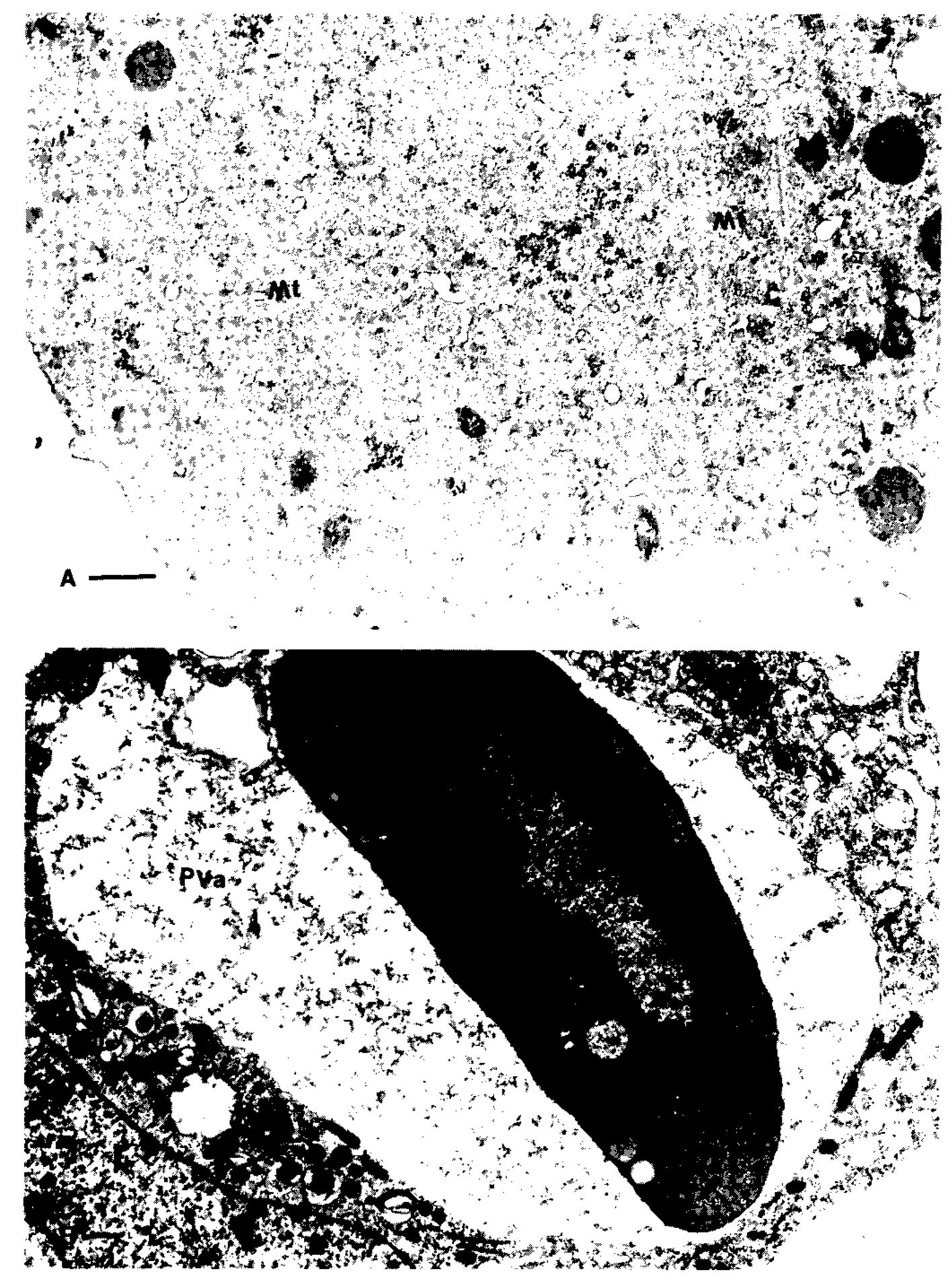


Fig. 9-A: coagulocyte – the arrows indicate labile granules. B: phagocytosis of a coagulocyte (CO) by a plasmatocyte. Ly\* = lysosomes in all directions; Mt = microtubules; N = nucleus; PVa = phagocytic vacuole. Bar = 1  $\mu m$ .

do not present granules. The nucleus is usually eccentric and does not show enlarged perinuclear cisternae. They are very rich in free ribosomes and mitochondria and poor in membranous systems. They are quite rare, always

less than the 2% of the total hemocyte number (Table II).

Adipohemocytes (Figs 11 and 12): round or oval, very large hemocytes (24-90  $\mu$ m). The

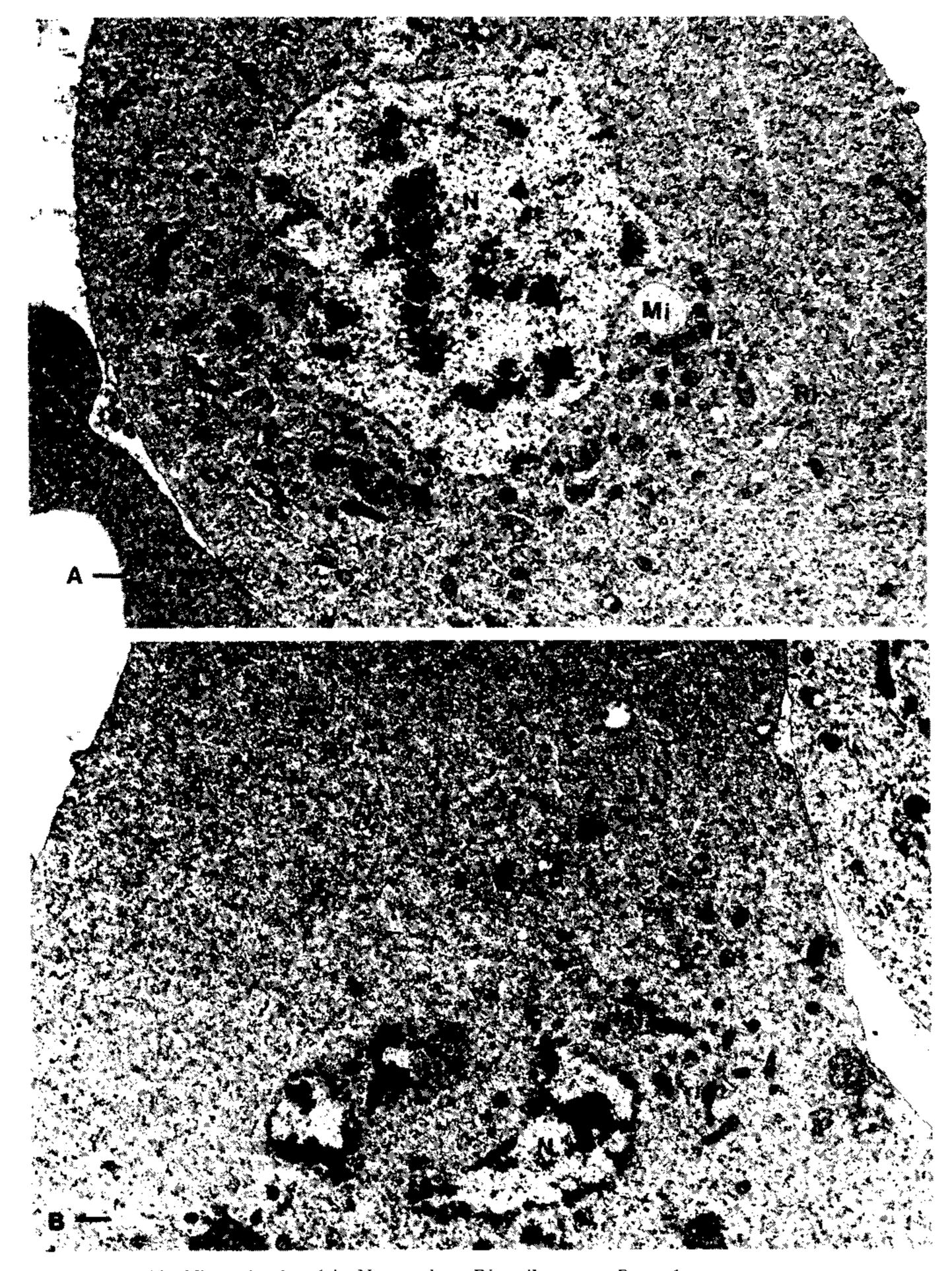


Fig. 10: oenocytoids. Mi = mitochondria; N = nucleus; Ri = ribosomes. Bar = 1  $\mu$ m.

small nucleus (6-9  $\mu$ m) is usually eccentric and nucleoli are frequently seen. The cytoplasm is mainly characterized by a large amount of small to large lipid droplets. They are strongly stained by the Sudan Black B (Barracco et al., 1987) which confirms the presence of lipids. The

cytoplasm is also rich in glycogen and contains a well-developed RER, Golgi bodies and several large mitochondria.

These hemocytes are not always present in circulation and look like free fat body cells

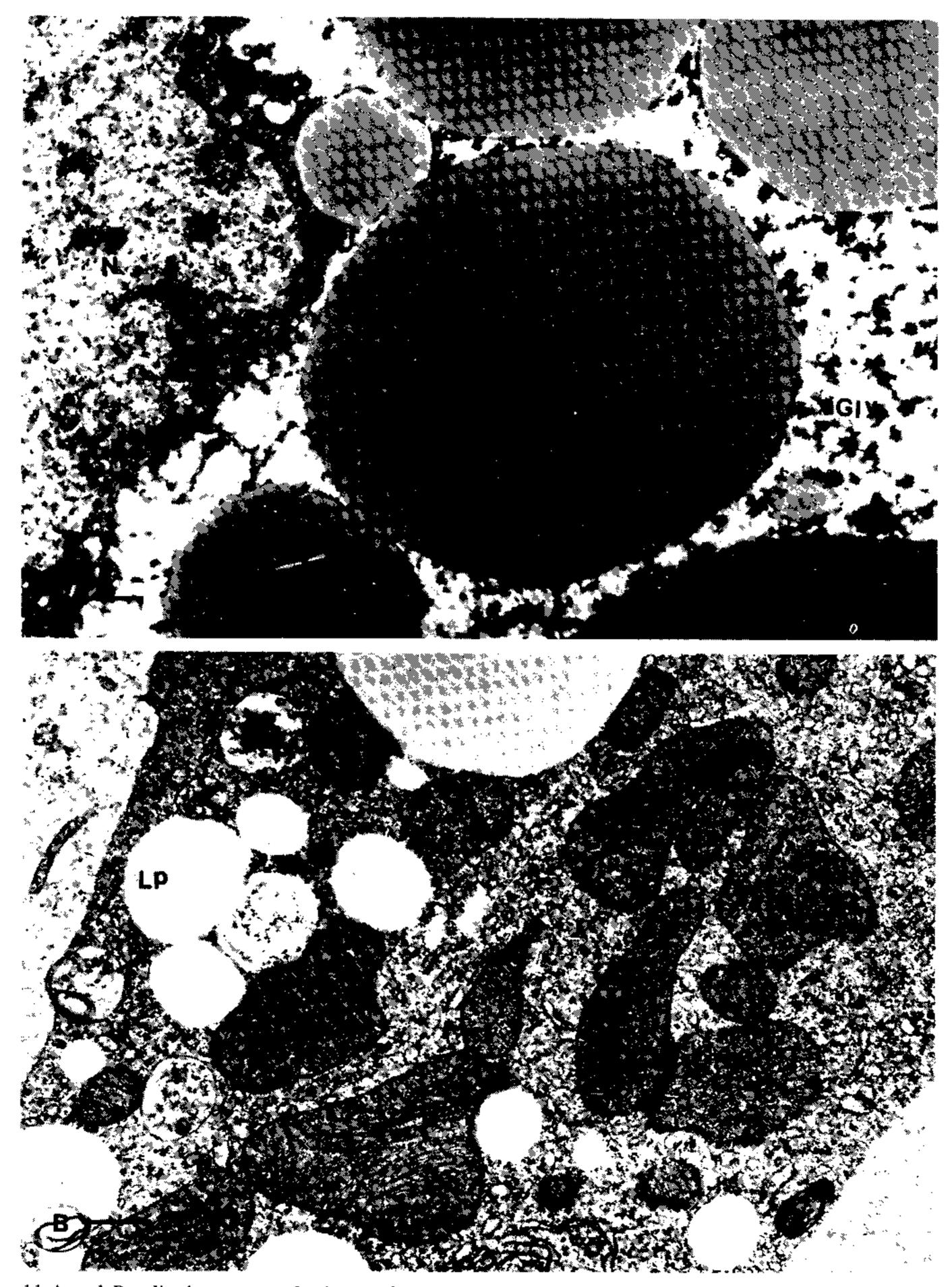


Fig. 11-A and B: adipohemocytes. In A, we observe large lipid droplets (Lp) and glycogen (Gly). In B, another cell portion containing several large mitochondria (Mi). N = nucleus. Bar = 1  $\mu$ m.

which entered the hemolymph. However, some of them, are not as large as it would be expected for true fat cells.

### DISCUSSION

In a previous paper (Barracco et al., 1987),

we described the hemocytes of *P. megistus*, using phase contrast microscopy and histochemical tests. The present TEM observations confirm, in several aspects, our previous results. As already observed through light microscopy, prohemocytes and plasmatocytes are quite

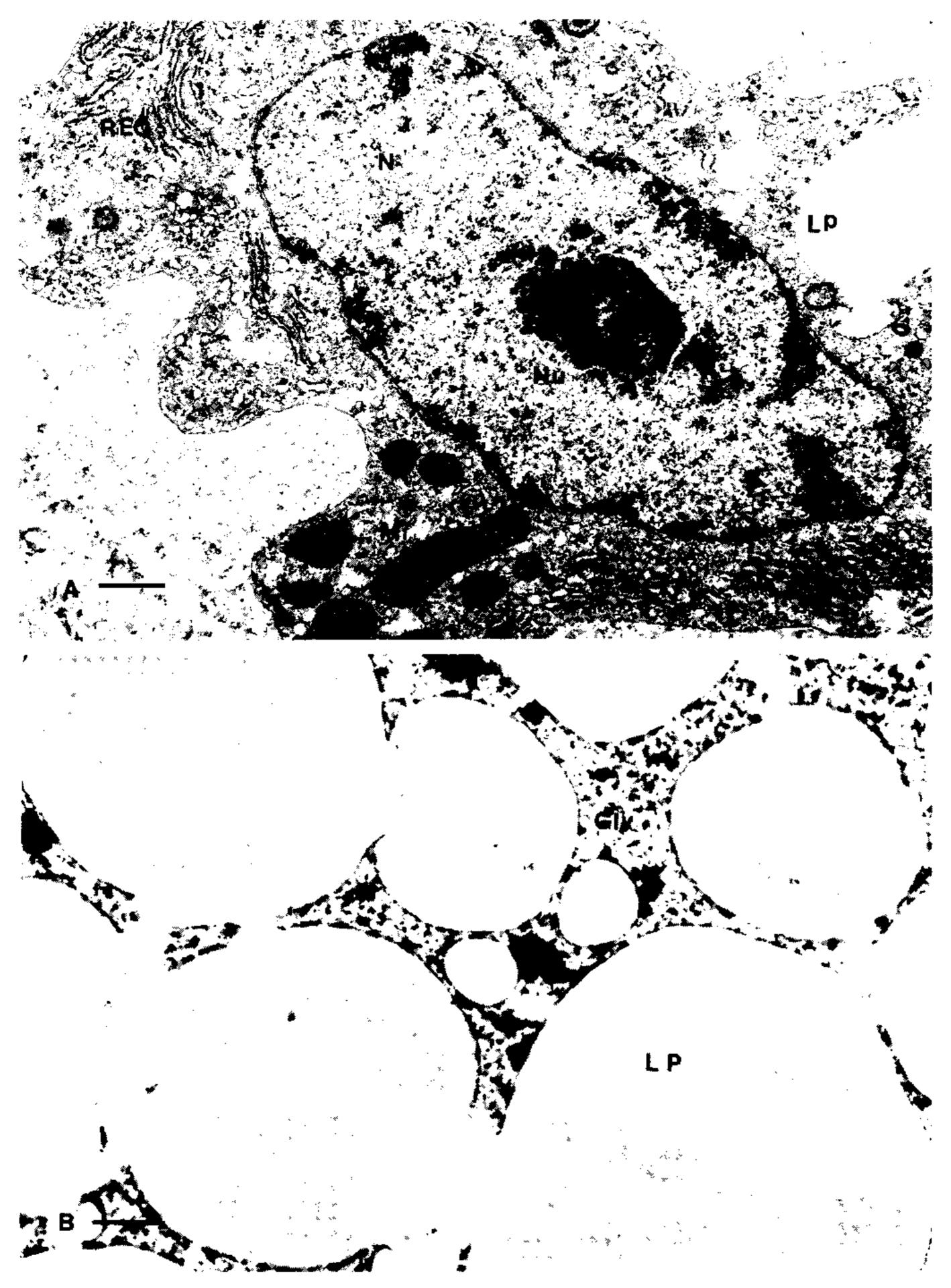


Fig. 12-A and B: adipohemocytes. In A, we observe a cell portion containing the nucleus (N) and a large amount of REG. In B, another portion of the same cell, containing many lipid droplets (Lp) of different sizes. Gly = glycogen; Mi = mitochondria; Nu = nucleulus. Bar = 1  $\mu$ m.

characteristic and do not raise classification doubts. Prohemocytes are the most indifferentiated cells and probably originate plasmatocytes by maturation. The presence of intermediate forms between them (Fig. 2-A and B)

strongly suggests this differentiation. Plasmatocytes are much richer in membranous systems and are especially phagocytic hemocytes. They contain a large amount of lysosomes, as previously confirmed by the Gomori method for

TABLE II
Differential hemocyte counts (DHC) during the development of Panstrongylus megistus expressed as relative
percentages

of	Number of cells counted	% -	Hemocyte types				Takit	
			PR	PL + GR**	СО	OE	AD	Total
4th instar	2057	Mean variation	10.9 4.3-20.6	52.4 36.4-66.1	27.8 15.9-46.9	1.5 0.8-3.8	7.5 0-27.5	100
5th instar	2025	Mean variation	9.9 3.1-26.3	50.8 40.0-61.5	28.7 10.3-46.5	1.2 0-4.0	9.3 0-35.2	100
Adults	2113	Mean variation	9.5 4.2-16.0	43.2 18.2-67.5	45.8 22.5-61.8	1.4 0-5.8	0 -	100

For each stage we used a minimum number of 15 insects. These animals were sampled from the 6th to the 10th day of feeding. Adult males and females were sampled throughout the period occurring immediately after the last moult to the 3rd day after it.

PR: prohemocytes; PL: plasmatocytes; GR: granulocytes; CO: coagulocytes; OE: oenocytoids; AD: adipohemocytes.

abundance of lysosomes reflects their participation in phagocytosis. In fact, these cells may ingest an extremely large number of different biological particles, such as bacteria (Fig. 5-A and B), chicken erythrocytes and others (Barracco et al., 1987). Plasmatocytes are sometimes observed engulfing what we are now identifying as coagulocytes (Fig. 9-B) and previously called oenocytoids (Barracco et al., 1987). In R. prolixus Wigglesworth (1933; 1973) and Jones (1965) also pointed out their ingestion by plasmatocytes. The identification of plasmatocytes is, thus, simplified by the fact that they are the only hemocyte type which shows phagocytic capacity. However, Wigglesworth (1973) also denominated plasmatocytes, the granular cells in R. prolixus, which are involved in the formation of basement membranes. These granular cells correspond to what we are calling granulocytes. In this way, Wigglesworth did not separate plasmatocytes from granulocytes. On the other hand, Lai-Fook (1970) equally classified the granular hemocytes of R. prolixus as plasmatocytes. In P. megistus, TEM analyses easily allow the distinction of two hemocyte types with quite different ultrastructural features. Nevertheless, when observed by light microscopy, plasmatocytes are very similar to granulocytes, since they also have granules, sometimes. The cells, we denominate granulocytes contain many granules with different electrondensity degrees

acid phosphatase (Barracco et al., 1987). The (Fig. 7-A). Many of them show an internal structuration, similar to a system of micromicrotubules (Fig. 7-A and B), as already described for granulocytes of other insects species (see Gupta, 1979). Granulocytes are also very rich in RER, which shows different degrees of cisternae dilatation (Fig. 6-B). and Golgi complex. Both organelles are probably related to the synthesis of granules. Granulocytes are non phagocytic in contrast to plasmatocytes. We preferred to classify them, in two separate classes, since they are ultrastructurally distinguishable and also because they behave differently with respect to phagocytosis. Their classification in different types must be important for comparative studies with the hemocytes of other insect species.

> Concerning the coagulocytes, their classification is more controversial. In our previous paper (Barracco et al., 1987), we called them oenocytoids. However, after TEM analyses, we actually observed that they are morphologically more similar to coagulocytes, than to oenocytoids. In R. prolixus, this cell type was initially denominated oenocytoid by Wigglesworth (1933; 1955). In 1965, using the phase contrast microscope, Jones disagreed with Wigglesworth's classification and named them granulocytes. After TEM studies, Wigglesworth (1973; 1979) reconfirmed his original classification, as oenocytoids. On the other hand, still in R. prolixus, Lai-Fook (1970) deno-

<sup>\*\*</sup> Because of the difficulties to distinguish plasmatocytes from granulocytes, both hemocyte types were considered together.

minated these same cells granulocytophagous, based on a term previously introduced by Jones (1965) to identify plasmatocytes that engulfed granulocytes. The term granulocytophagous was also employed by Dorn (1979) in the hemipteran, Oncopeltus fasciatus. Although we first called these cells, oenocytoids, in P. megistus in agreement with Wigglesworth (1933; 1955; 1973; 1979), the present TEM analyses indicate that they are ultrastructurally similar to coagulocytes. Coagulocytes are usually described as fragile, hyaline unstable hemocytes combining the features of granulocytes and oenocytoids (see Gupta, 1979). They contain a small and usually eccentric nucleus which appears cartwheel-like under phase contrast, due to the arrangement of its chromatin. TEM analyses generally show swollen perinuclear cisternae (Gregoire & Goffinet, 1979) which distinguish these cells from the other types. Plasma membrane usually present microruptures. The cytoplasm contains little or no RER, small numbers of ribosomes and a variable number of granules (Rowley & Ratcliffe, 1981). In vitro these cells quickly degranulate, forming islets of coagulation around them (Gregoire, 1955). They were first called coagulocytes by Gregoire & Florkin (1950) because of their involvement in hemolymph coagulation. However, Jones (1954; 1962) preferred to name them cystocytes, since these cells are also present in insects whose hemolymph does not coagulate. In R. prolixus, Price & Ratcliffe (1974) reported the presence of cystocytes which certainly corresponded to the oenocytoids, granulocytes and granulocytophagous cells described in this same insect by Wigglesworth (1933; 1955; 1973), Jones (1965) and Lai-Fook (1970) respectively. The hemolymph of R. prolixus does not clot.

The coagulocytes of *P. megistus* have pronounced perinuclear cisternae which are observed only in this cell type. This feature, together with the presence of labile granules that are discharged in the hemolymph and the small amount of membranous systems strongly suggest that they must be coagulocytes. However, these hemocytes are not involved in coagulation mechanisms, since the hemolymph of *P. megistus* does not clot. However, as mentioned before, coagulocytes are not necessarily related to coagulation processes and this denomination is also employed for hemocytes which have particular morphological features.

Besides these hemocyte types, there is also a small number of other cells, that are considerably similar to the oenocytoids described for other insects (see Gupta, 1979). They are large in size and the basophilic, homogeneous and little electrondense cytoplasm is agranular and poor in membranous systems. They do not show pronounced perinuclear cisternae as the coagulocytes (Fig. 10-A and B). Jones (1965) called them oenocytoids in R. prolixus, while Wigglesworth (1933; 1955; 1973) described them as large agranular cells. On the other hand, in the same insect Lai-Fook (1970) through TEM analyses, classified the large spindle-shaped, granular-free hemocytes, very poor in RER, as oenocytoids. They certainly correspond to what we are calling oenocytoids in P. megistus.

With respect to the adipohemocytes, ultrastructural observations confirm that they are very similar to fat body cells, as previously described (Barracco et al., 1987). Adipohemocytes are characteristically rich in lipid inclusions of different sizes and contain large amounts of glycogen (Fig. 11-B and 12-B). The authors who studied these hemocytes on R. prolixus, believe that they may be free fat body cells which entered the hemolymph. This can be also true for P. megistus.

The main investigations on hemocyte population dynamics during the developmental stages of reduviids, were performed by Jones & Liu (1961) and Jones (1962; 1967) in R. prolixus. Other hemipterans have also been investigated such as O. fasciatus (Feir, 1964; Feir & O'Connor, 1969) and Halys dentata (Bahadur & Pathak, 1971). Jones & Liu (1961) found 800 to 2,000 hemocytes/mm<sup>3</sup> of hemolymph in R. prolixus. In O. fasciatus Feir (1964) reported a range of 200 to 3,600 cells/mm<sup>3</sup> along its development. However, using more sophisticated methods, Feir & O'Connor (1969) found much larger values (20,000 to 40,000 hemocytes/mm<sup>3</sup>) in 5th instar nymphs of the same insect. In H. dentata Bahadur & Pathak (1971) described a gradual increase of THC during the insect development, varying from 400 hemocytes/mm<sup>3</sup> in the 2nd instar up to about 1,500 cells/mm<sup>3</sup> in the adults. In this insect, the THC decreases very little before ecdysis, strongly declines immediately after it, and, increases significantly again, during the nymphal period. Wigglesworth (1955) observed in R. prolixus that THC increases just before

ecdysis, decreases during it, and increases again immediately after the moult.

THC was estimated in the 4th, 5th instar nymphs and adults of *P. megistus* and revealed higher values than in *R. prolixus*. There is an increase of THC from the 4th to the 5th instar followed by a significant decrease in adults (Table I).

In R. prolixus, Jones (1967) studied the hematological variation of 4th and 5th instar nymphs and male and female adults in relation to ecdysis and feeding period. He observed that during the starvation period, that follows ecdysis, plasmatocytes usually increased in number, while granulocytes (= coagulocytes) decreased. After feeding, however, the reverse is observed. In adults, the plasmatocytes increased again in number and coagulocytes decreased. In R. prolixus we can easily deduce that plasmatocytes and coagulocytes are the predominant cell types. Plasmatocytes may represent up to 90% of the total hemocyte number in adults after ecdysis and coagulocytes may reach 84% in adults after feeding. Prohemocytes and oenocytoids occur always in reduced numbers. In his analyses, Jones also described fat-cells, which included adipohemocytes, even though these cells were always very scarce.

As can be inferred from Table II, plasmatocytes plus granulocytes in P. megistus, are the predominant cell types in nymphs, contributing with at least 50% of total hemocyte number. Coagulocytes are the second more numerous performing around 25% of the total hemocyte number. These results are, thus, similar to those obtained for R. prolixus (Jones, 1967), where these hemocyte types are also predominant. However, an opposite pattern occurs when adults of P. megistus are compared with R. prolixus. In those insects, there is a significant increase of coagulocytes, that may reach 46% of the total hemocyte number, and a concomitant decrease of plasmatocytes (43%) (Table II). Prohemocytes and oenocytoids of *P. megistus* are always scarce and practically do not change during the insect development (Table II) similarly to what happens to R. prolixus.

In *P. megistus*, adipohemocytes seem to be more abundant than in *R. prolixus*, where they are very scarce (Jones, 1967). In the 5th instar nymphs, adipohemocytes may reach 35% of

total hemocyte numbers (Table II). However, fat cells are never observed in adults (Table II) and they are not always present in *P. megistus* nymphs. As previously discussed, even though adipohemocytes of *P. megistus* are frequently found in the hemolymph of nymphs, we do not exclude the possibility that this cell type may correspond to free fat body cells which entered the hemolymph.

## **RESUMO**

Ultra-estrutura dos hemócitos de Panstrongylus megistus (Hemiptera: Reduviidae) — Estudos ao microscópio eletrônico de transmissão revelaram a presença de seis tipos de hemócitos na hemolinfa de Panstrongylus megistus. Estes resultados confirmam parcialmente os obtidos anteriormente através da microscopia de luz. Pró-hemócitos: células pequenas e arredondadas, cuja delgada faixa citoplasmática é especialmente rica em ribossomos livres e pobre em sistemas membranosos. Plasmatócitos: células polimórficas, cujo citoplasma caracteriza-se por um retículo endoplasmático rugoso (RER) bem desenvolvido e principalmente pela sua abundância em lisossomos. São células tipicamente fagocitárias. Algumas vezes, seu citoplasma mostra-se extremamente vacuolizado. Granulócitos: hemócitos granulares, cujos grânulos mostram diferentes graus de eletrondensidade e podem ou não apresentar estruturação interna. Coagulócitos: hemócitos ovalados ou fusiformes que se caracterizam pela presença de acentuadas cisternas perinucleares. O citoplasma é geralmente eletrondenso, pobre em organelas membranosas e contêm grânulos frágeis. Enocitóides: hemócitos grandes e muito estáveis em sua morfologia. Seu citoplasma é homogêneo, rico em ribossomos livres e pobre em sistemas membranosos. Adipo-hemócitos: células grandes que apresentam inclusões lipídicas características. Seu citoplasma é também rico em glicogênio. RER e mitocôndrias grandes.

As contagens totais (THC) e diferenciais (DHC) de hemócitos foram também calculadas. A THC cresce de 2.900 hemócitos/mm³ de hemolinfa em ninfas de 4º estágio para 4.350 em ninfas de 5º estágio. Há, no entanto, um decréscimo significativo nos adultos, onde a THC passa para 1.950 hemócitos/mm³ de hemolinfa. Plasmatócitos e coagulócitos são os hemócitos predominantes em P. megistus.

Palavras-chave: hemócito — Panstrongylus megistus — ultra-estrutura

### **ACKNOWLEDGMENTS**

To Dr P. Abrahamsom, Dr J. A. Bauer and to G. Ferreira de Lima for the use of the electronic microscope of the Department of Histology and Embriology of ICB-USP. To M. Steindel and Dr B. Schlemper Jr for supplying the cultures of triatomines and to Dr N. Ferrari for English revision of the manuscript.

#### REFERENCES

- BAHADUR, J. & PATHAK, J. P. N., 1971. Changes in the total hemocyte counts of the bug, *Halys dentata* under certain conditions. *J. Insect Physiol.*, 17: 329-334.
- BARRACCO, M. A.; OLIVEIRA, R. & SCHLEMPER JR, B., 1987. The hemocytes of *Panstrongylus megistus* (Hemiptera: Reduviidae). *Mem. Inst. Oswaldo Cruz*, 82:431-438.
- DORN, A., 1979. Ultrastructure of differentiating hemocytes in the embryo of *Oncopeltus fasciatus* (Insecta: Heteroptera). *Cell Tissue Res.*, 187: 479-488.
- FEIR, D., 1964. Hemocyte counts of the large milkweed bug, *Oncopeltus fasciatus*. *Nature*, 202: 1136-1137.
- FEIR, D. & O'CONNOR JR, G. M., 1969. Liquid nitrogen fixation: A new method for hemocyte counts and mitotic indices in tissue sections. Ann. Entomol. Soc. Amer., 62: 246-249.
- GREGOIRE, Ch., 1955. Blood coagulation in Arthropods. V. Studies on hemolymph coagulation in 420 species of insects. *Arch. Biol.*, 66: 103-148.
- GREGOIRE, Ch. & FLORKIN, M., 1950. Blood coagulation in Arthropods. I. The coagulation of insect blood, as studied with the phase contrast microscope. *Physiol. Comp. Oecol.*, 2: 126-139.
- GREGOIRE, Ch. & GOFFINET, G., 1979. Controversies about the coagulocyte, p. 189-229. In: A. P. Gupta, *Insect hemocytes*. Cambridge University Press, Cambridge.
- GUPTA, A. P., 1979. Structure and taxonomic signifi-

- cance, p. 85-128. In: A. P. Gupta, *Insect hemo-cytes*. Cambridge University Press, Cambridge.
- JONES, J. C., 1954. A study of mealworm hemocytes with phase contrast microscopy. *Ann. Entomol. Soc. Amer.*, 47: 308-315.
- JONES, J. C., 1962. Current concepts concerning insect hemocytes. Amer. Zool., 2: 209-246.
- JONES, J. C., 1965. The hemocytes of Rhodnius prolixus. Biol. Bull. Woods Hole., 129: 282-294.
- JONES, J. C., 1967. Normal differential counts of hemocytes in relation to ecdysis and feeding in Rhodnius. J. Insect Physiol., 13: 1133-1141.
- JONES, J. C. & LIU, D. P., 1961. Total and differential hemocyte counts of *Rhodnius prolixus* Stal. *Bull. Entomol. Soc. Amer.*, 7: 166.
- LAI-FOOK, J., 1968. The fine structure of wound repair in an insect (Rhodnius prolixus). J. Morphol., 124: 37-78.
- LAI-FOOK, J., 1970. Haemocytes in the repair of wounds in an insect (Rhodnius prolixus). J. Morphol., 130: 297-314.
- PRICE, C. D. & RATCLIFFE, N. A., 1974. A reappraisal of insect hemocyte classification by the examination of blood from fifteen insect orders. Z. Zellforsch. Mikrosk. Anat., 147: 537-549.
- ROWLEY, A. F. & RATCLIFFE, N. A., 1981. Insects, p. 421-488. In A. G. Gupta, *Invertebrate Blood Cells. Vol. II*. Academic Press, London.
- TAUBER, O. E. & YEAGER, J. F., 1935. On the total hemolymph (blood) counts of insects. I. Orthoptera, Odonata, Hemiptera, and Homoptera. Ann. Entomol. Soc. Amer., 28: 229-240.
- WIGGLESWORTH, V. B., 1933. The physiology of the cuticle and of ecdysis in *Rhodnius prolixus* (Triatomidae: Hemiptera) with special reference to the function of the oenocytes and of the dermal glands. Q. J. Microsc. Sci., 76: 269-318.
- WIGGLESWORTH, V. B., 1955. The role of the hemocytes in the growth and moulting of an insect, *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol.*, 32: 649-663.
- WIGGLESWORTH, V. B., 1973. Haemocytes and basement membrane formation in *Rhodnius*. J. Insect Physiol., 19: 831-844.
- WIGGLESWORTH, V. B., 1979. Hemocytes and growth in insects, p. 303-318. In: A. P. Gupta, *Insect Hemocytes*. Cambridge University press, Cambridge.