 Cytologia **67**: 301–308, 2002

The Origin of Lipid Droplets in the Post-pharyngeal Gland of *Dinoponera australis* (Formicidae: Ponerinae)

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Received May 15, 2002; accepted June 17, 2002

Summary The post-pharyngeal gland of normal and starvation ants was studied under TEM. This study showed an orgin of lipids droplets from mitochondria (named derivate mitochondria) in the normal ants and the lipids absence or reduction, in fasting ants.
Key words PPG, Lipid droplets, *Dinoponera*, Ants, Mitochondria.

The post-pharyngeal gland constitutes part of the salivary system and it is typical of ants. Until now, the functions attributed to this gland are the production of the odor substance characteristic of the colony, and being also a diverticulus of the digestive tract (Delage-Darchen 1976, Gama 1985, Soroker *et al.* 1994, 1995, Caetano 1998).

Mitochondria are present throughout the cytoplasm of this gland's cells. In most of the cases, their distribution cannot be associated to the flux of substances inside the cell, since they are concentrated at both the apex as well as the base of the cell and are scarce in the area surrounding the nucleus (Caetano 1998). According to Billen (1991), insect's glands that produce pheromones are rich in mitochondria. Zylberber *et al.* (1974) proposed that mitochondria are not a part of the membrane reposition system.

Falco (1992) detected a large amount of elongated mitochondria in the post-pharyngeal gland of *Camponotus rufipes*. In addition, Schoeters and Billen (1997) described mitochondria with different shape and sizes in *Dinoponera quadriceps* and *D. australis*. Quennedey (1998) described the large amount of mitochondria with various shapes as one of the main characteristics of type I secretion cells. This type of secretion cells comprises the post-pharyngeal gland of ants (Holldobler and Wilson 1990).

The cells of the post-pharyngeal gland of ants are generally rich in neutral lipid droplets. The presence of these droplets have always been associated to food capture, since they can be found in the gland's lumen immediately after feeding and, afterwards, they are found in the cuticular epithelium (Zylberberg *et al.* 1974). Gama (1985) showed the presence of 2 types of lipid droplets in a cell from the post-pharyngeal gland of *Camponotus rufipes*, the smaller were electron-lucid and the bigger have electron-dense content with granular edge; both were located at the medium-basal portion of the cell.

The lipid droplets may be visualized since the seventh day of *C. rufipes*' adult life, reaching its maximum at the 21st day, and its maintained in this fashion until the 60th day of life. In parasite-infected individuals, this compound is present in lesser amounts than in healthy ones (Falco 1992). According to Schoeters and Billen (1997), the amount of this compound present in the post-pharyngeal gland of *D. quadricens* and *D. australis* depends on the ant's age.

The association between mitochondria and the origin of the lipid droplets in insects was first suggested by Ranade (1933), who proposed that the lipids present in the vitellum were originated

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from transformed mitochondria. Boissin (1970) described the presence of mitochondria without crest and with lipid droplets adhered to them in pseudoscorpions.

The present work shows the formation of lipid droplets from and into mitochondria in the post-pharyngeal gland of *Dinoponera australis.*

Materials and methods

The post-pharyngeal glands were obtained by dissection of the head of normal and starvation (8 d fast) ants *D. australis.* These glands were subjected to fixation with modified Karnovsky solution during 2 h. Afterwards, we followed the routine preparation for transmission electron microscopy, which consisted on infiltration with a mixture of epon–araldite (1 : 1) during 6 h, embedding in pure resin during a period of 12 to 24 h at room temperature, and final polymerization at 60°C during 24 h.

Ultra-thin sections were obtained with a diamond blade using a Reichter ultramicrotome. The sections were stained with both lead citrate and uranyl acetate and analysed under a TEM CM 100 Phillips UNESP, campus in Rio Claro SP, Brazil.

Results and discussion

There is a large amount of mitochondria in the cells of the normal post-pharyngeal gland of *D. australis* and their distribution is regular throughout the cytoplasm, although they are at a higher concentration at basal and apical cytoplasm. The mitochondria show various shapes, from elongated to circular (Fig. 1). The lipid droplets are relatively abundant, concentrate at the basal region (Figs. 2, 3).

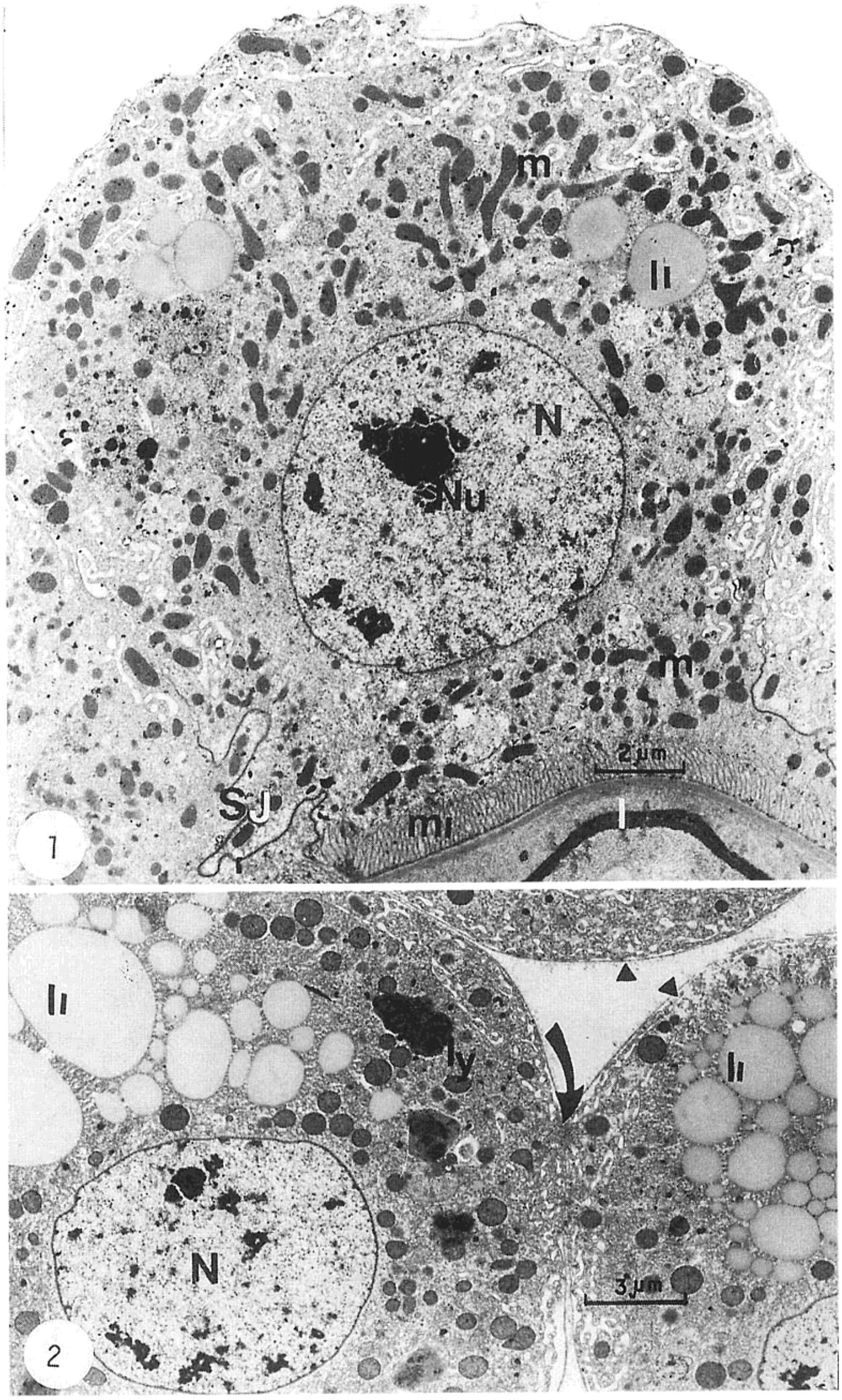
According to Zylberberg *et al.* (1974), the mitochondria are the most abundant organelle in the cytoplasm of the post-pharyngeal gland's cells. Falco (1992) described many elongated mitochondria in the post-pharyngeal gland of *C. rufipes.* In addition, the post-pharyngeal gland of *Dinoponera quadriceps* and *D. australis* had abundant mitochondria with a wide variety of shapes and sizes, as demonstrated by Schoeters and Billen (1997).

Our results are in agreement with the works cited above. However, we also verify that most of the mitochondria are in a division process (Figs. 4, 5), which means that this organelle undergoes an accentuated reposition process, as the pre-existent mitochondria wear down and/or convert into other forms.

According to Billen (1991), pheromones-secreting glands are rich in elongated mitochondria. Ratcliffe and King (1969) showed that there were alterations in the number and shape of mitochondria found in the wasp *Nasonia vitripennis* when subjected to starvation.

In *D. australis* we observed several electron-dense structures, showing an internal membrane arrangement in one of the ends, similar to tiny reticules (Figs. 6, 7). A description of such structures does not exist in the literature, however, Ratcliffe and King (1969) related the transformation of mitochondria into secondary lysosomes or autophagic vacuoles before they form lipid droplets.

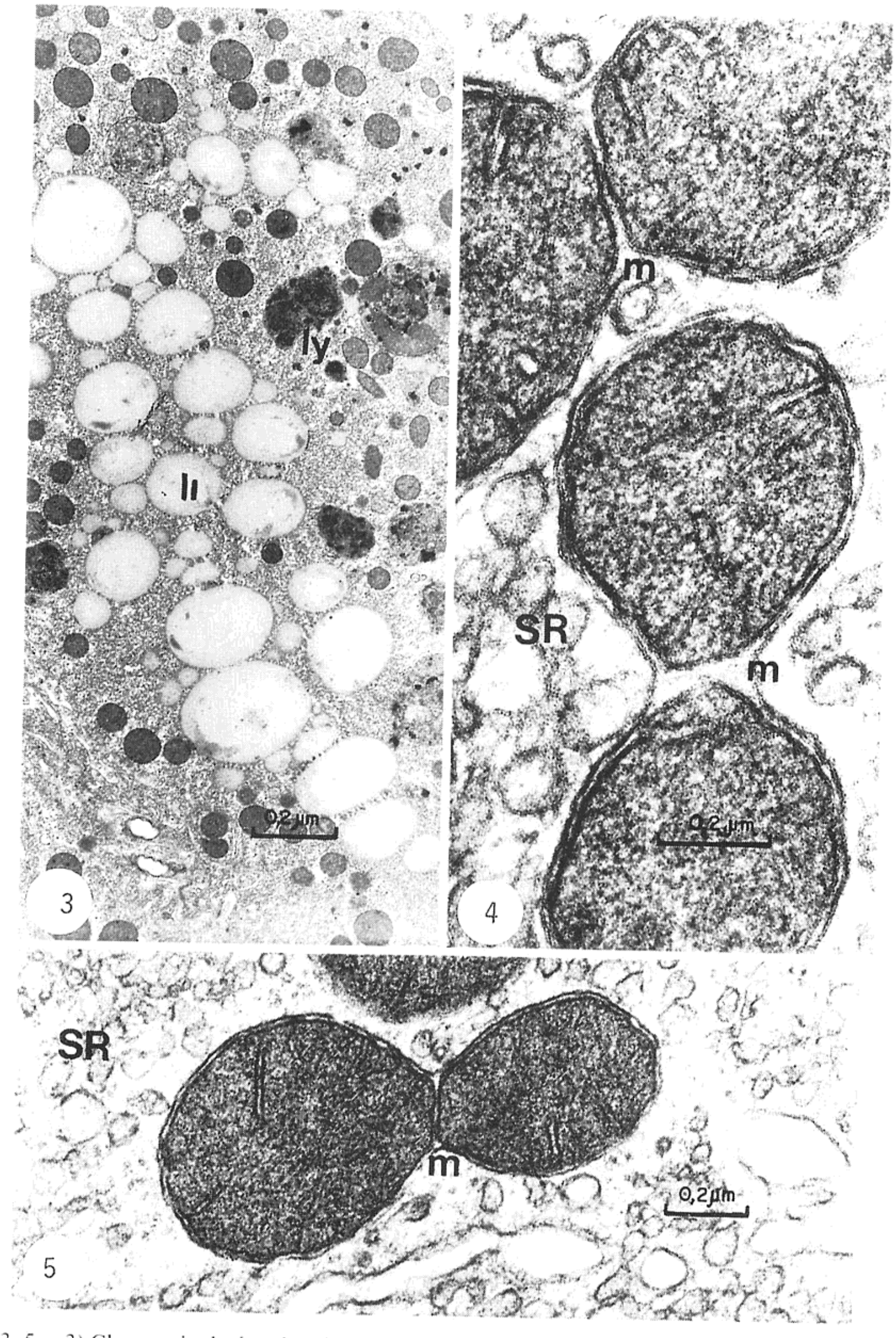
The steps of this transformation can be observed in Figs. 8–11. In Fig. 8 it is possible to note, laying side by side, a lipid droplet at the beginning of formation and another at the final stage, the latter is bigger than the rest of the mitochondria. These kinds of structures are abundant in the cytoplasm of cells of the post-pharyngeal gland of *D. australis.* However, the Caetano (1998) does not believe that they are responsible for all the lipids present in the gland, since it is known that these cells absorb lipid from the lumen, as was demonstrated by Zylberberg *et al.* (1974), even though Schoeters and Billen (1997) stated that the contents of this gland in *D. quadriceps* and *D. australis* did not include lipids. It is also widely known that there are 2 kinds of lipids in the cells from the post-pharyngeal gland of *Camponotus rufipes* (Gama 1985). Probably, one of them could be of mi-



Figs. 1, 2. 1) General view of post-pharyngeal gland cell, showing many mitochondria shapes (m). N=nucleus, Nu=nucleolus, li=lipids droplets, mi=mirovilli, l=lumen, SJ=septate junction. 2) Two cells with many lipids droplets (li). ly=lysosome; gap junction (arrow); basal memberane infold (head arrow).

tochondrial origin.

In individuals submitted to starvation for 8 days, the lipid droplets are scarce and more common at the basal cytoplasm. These droplets are of reduced size. The mitochondria, though abundant, are smaller and circular, being frequently found at the basal portion of the cell; some of them assuming a ring shape (Figs. 12–14). Derivative mitochondria, which are very abundant in the post-pharyngeal gland's cells of normal individuals, are rare or absent in those subjected to starvation

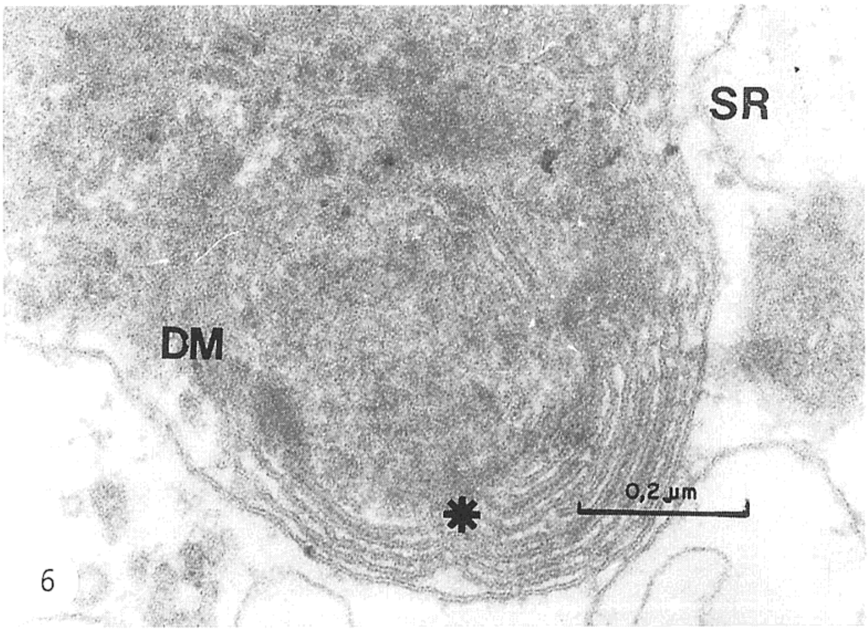


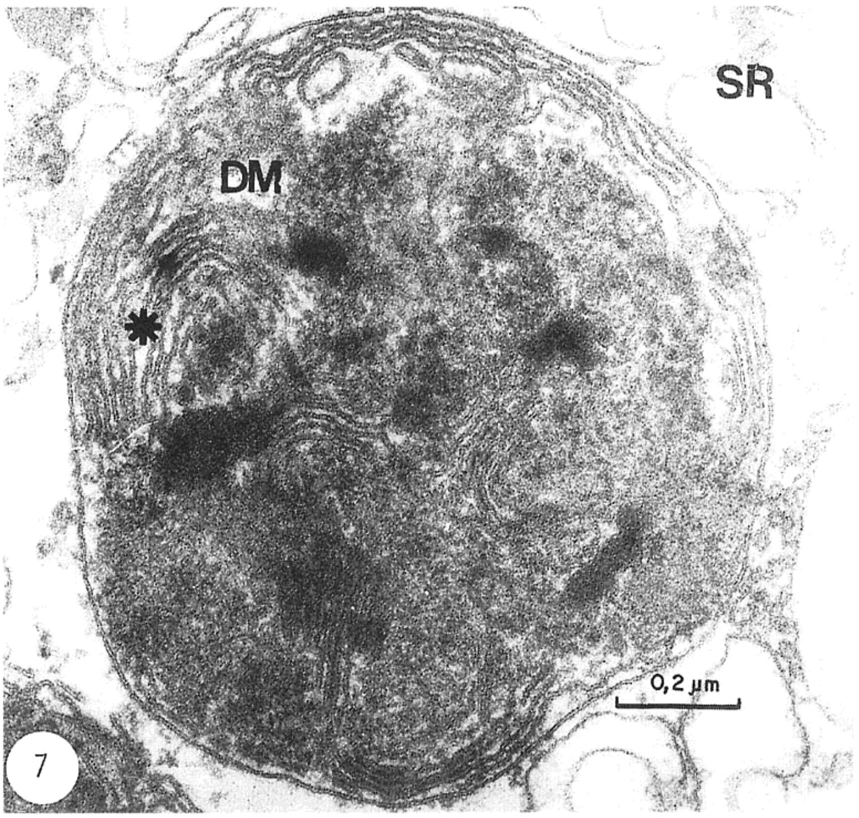
Figs. 3–5. 3) Close up in the basal region with many lipids droplets and lysosome (ly). 4, 5) Mitochondria (m) in division process. SR=smooth endoplasmic reticule.

and show several levels of electron-density. Lysosomes and peroxisomes are abundant in post-pharyngeal gland's cells of 8 day fast ants.

In insects subjected to starvation, alterations on both the amount of lipid droplets and on the number and shape of mitochondria were first described by Ratcliffe and King (1969) in the wasp *N. vitripennis*, it was suggested that the mitochondria uptake portions of the cytoplasm and rough endoplasmic reticule originates the lipid droplet inside it.

Similar event can be observed in *D. australis*, since in individual subjected to starvation the amount of smooth endoplasmic reticule diminishes drastically. This reticule could have been used for the production of lipids. Consequently, part of the lipids found in the cells of the post-pharyngeal gland of *D. australis* was originated from the derivative mitochondria, which afterwards

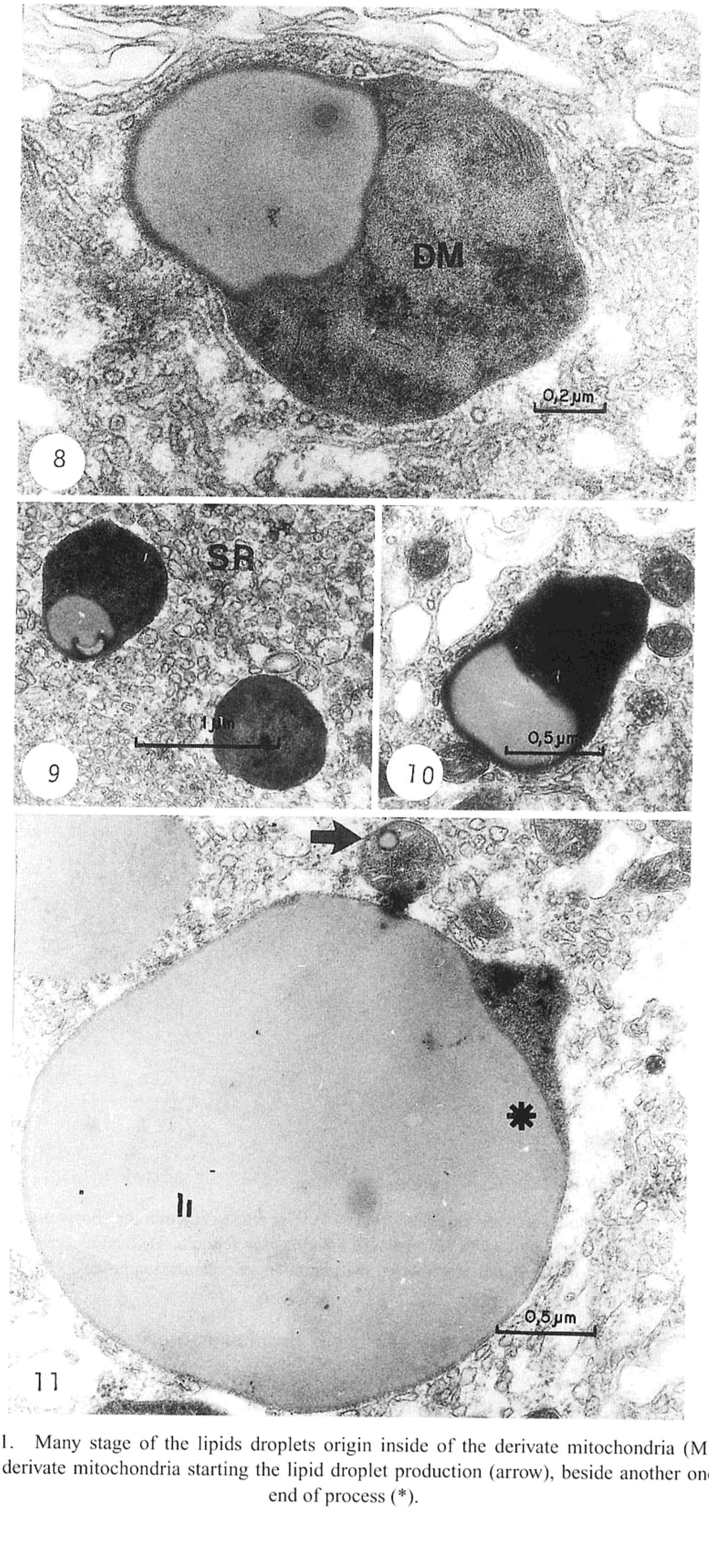




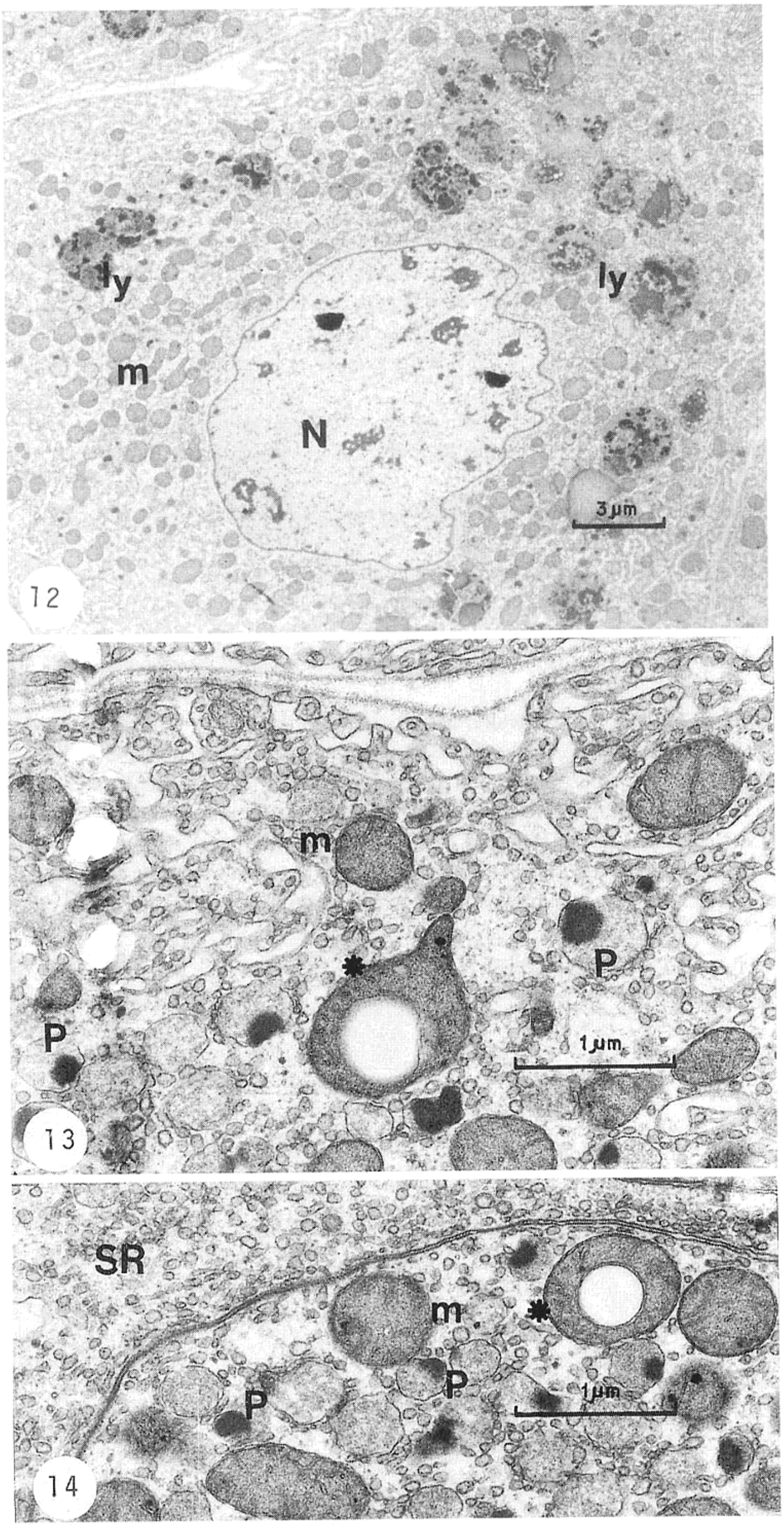
Figs. 6, 7. 6) Close up in derivate mitochondria (DM). The internal membrane shows organization like endoplasmic reticule organization (*). SR=smooth endoplasmic reticule. 7) General view a derivate mitochondria (DM) with the internal membrane in an organization process (*).

formed the lipid droplet, in starvation ants the lipids droplets can originate from the smooth endoplasmic reticule.

Therefore, besides those already documented (Alberts *et al.* 1997) we add another function for the mitochondria in insects: the production of lipids, just as was previously suggested by other authors, such as Ranade (1933), Ratcliffe and King (1969), Boissin (1970), and Camargo-Mathias (1993), although only Ratcliffe and King showed some figures that confirmed their suppositions.



Figs. 8–11. Many stage of the lipids droplets origin inside of the derivate mitochondria (MD). 11) there is a derivate mitochondria starting the lipid droplet production (arrow), beside another one at the end of process (*).



Figs. 12–14. Many aspect of the post-pharyngeal gland from starvation (8 days fast) ants. 12) we can seen many lysosomes (ly), few lipids droplets and no derivate mitochondria. 13, 14) we can see many mitochondria (m) among peroxisomes (P) and some ring shape mitochondria (*).

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