

Vitellogenesis in *Varroa jacobsoni*, a parasite of honey bees

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ABSTRACT

Reproduction in *Varroa jacobsoni* occurs only in cells of the capped honey bee brood. Female mites were sampled at different times after cell sealing and ovaries containing a vitellogenic oocyte of the first gonocycle were examined under an electron microscope. It was found that the cytoplasmic connection between the lyrate organ and the oocyte persists far into the vitellogenic growth phase. In addition, a large amount of yolk material is taken up from the haemolymph. All ultrastructural features characteristic of vitellogenesis, such as microvilli, coated pits, vesicles and growing yolk platelets, are present. If more than four *Varroa* females live in an overcrowded brood cell, they appear to be in stress conditions and their vitellogenic oocytes may become atretic. Alterations typical for oocyte degradation and oosorption were observed in such situations.

Key words: *Varroa*, bee parasite, ultrastructure, vitellogenesis, oosorption.

INTRODUCTION

The mite *Varroa jacobsoni*, a common and tolerated parasite of the Asian honey bee, *Apis cerana*, was recently transferred to the western hive bee, *Apis mellifera*. Because of possible unlimited propagation in colonies of this new host, varroatosis is causing severe world-wide problems for beekeepers (Matheson, 1994). Reproduction of the parasite is restricted to the short capped brood period during honey bee development. Within this limited time frame, female mites are capable of producing four to eight eggs. The first oviposition occurs approximately 3 days after cell capping and every subsequent day another egg is laid (Ifantidis and Rosenkranz, 1988). This sequence evidently requires a rather accelerated mode of oogenesis and embryogenesis with partly overlapping gonocycles (Steiner *et al.*, 1994). A trophic cord connecting the oocyte with the lyrate organ

was found to supply mitochondria and other organelles. How long this nursing strand functions, and how the ultrastructure of the oocyte is modified for uptake of exogenous yolk material, was investigated by electron microscopy. The results of such a study should help us to understand which functional adaptations are required for the rapid development of the oocyte. Only the pre-vitellogenic growth phase of the oocyte has so far been explored by electron microscopy (Alberti and Zeck-Kapp, 1986).

In addition, a brief description is given on oocyte atresia. Degeneration of unfertilized egg cells was reported in *Caloglyphus mycophagus* (Heinemann and Hughes, 1970) and in unmated argasid ticks (Diehl *et al.*, 1982). In *V. jacobsoni*, oosorption may be induced because of overcrowding in the cells of a honey bee brood. A single brood cell may be invaded by several female mites. This can lead to stress conditions (Donzé, 1995).

MATERIAL AND METHODS

Females of *V. jacobsoni* were collected from colonies of *Apis mellifera carnica* at apiaries in Bern-Liebefeld and in Tübingen. Ovaries were dissected and fixed for 2 h at room temperature in 2.5% glutaraldehyde in 0.09 M cacodylate buffer, pH 7.4, with 2.5% sucrose, washed in 0.18 M cacodylate buffer with 7.5% sucrose, post-fixed in 1% osmium tetroxide in 0.09 M cacodylate buffer, dehydrated in graded acetone concentrations and embedded in low-viscosity resin (Spurr, 1969). The ovaries were serially sectioned using a Reichert-Jung Ultracut E microtome. Semi-thin (0.50 μm) sections were stained with Toluidine blue (1%) and examined with an Olympus light microscope Vanox S AH-2. Ultra thin (60–90 nm) sections were contrasted with uranyl acetate and lead citrate and analysed in a Philips EM 201 transmission electron microscope. The reaction for the demonstration of polysaccharides (PAS) was applied according to Böck (1984).

RESULTS

Based on the proposed staging by Alberti and Zeck-Kapp (1986) for *Varroa* oogenesis, we used stage 4 for early and stage 5 for late vitellogenic oocytes. At early stage 4, the oocyte measured approximately 50 μm in diameter and approximately 200 μm in diameter at late stage 5.

During the vitellogenic phase, the oocyte protruded far into the haemocoel. The oocyte remained connected with the lyrate organ via the nutritive cord which measured approximately $5\ \mu\text{m}$ in diameter (Fig. 1a, b and d). Between the base of the oocyte and the ovary, one or two somatic cells surrounded the nutritive cord (Fig. 1b). Cytoskeletal elements, presumably microfilaments, maintained the cord shape (Fig. 1c). Many ribosomes were observed between a longitudinal array of microfilaments which suggested transport from the lyrate organ into the ooplasm. During stage 5 vitellogenesis, mitochondria could no longer be observed in the cord. In the region facing the somatic cells and after stage 4 has been initiated, a microfibrillar layer of approximately $0.1\ \mu\text{m}$ thickness was found in the cortical ooplasm that probably supported the enlarging vitellogenic oocyte mechanically (Fig. 1d). In this layer, the nutritive cord passed through a hole (Fig. 1b and d).

A thin basement lamina covered not only the surface of the protruding vitellogenic oocyte (Fig. 2a), but also the entire female reproductive organs including the spermatheca. The oocyte membrane underwent structural changes after initiation of vitellogenesis. Microvilli were formed in the perioocytic space beneath the basement lamina (Fig. 2a). Here, endocytosis occurred with the formation of numerous clathrin-coated pits and vesicles (Fig. 2c and d). Within the ooplasm, newly incorporated vesicles fused and enlarged in size. During the process of yolk accumulation, the protein material became condensed. In the early vitellogenic stage, oocyte yolk vesicles measured approximately $0.8\ \mu\text{m}$ in diameter. At the end of vitellogenesis, the enlarged yolk platelets were up to $9\ \mu\text{m}$ in diameter in the centre of the oocyte.

In addition to yolk incorporation, other reserve materials and organelles accumulated during the vitellogenic phase. Numerous mitochondria, free ribosomes as well as rough endoplasmic reticulum, Golgi complexes (Fig. 2b) and lipid droplets were seen in the ooplasm. In early stages of vitellogenesis, scattered deposits of glycogen were visible (Fig. 1b). Subsequently, areas with glycogen became abundant, often associated with lipid vacuoles (Fig. 1a).

The germinal vesicle contained one or two prominent nucleoli (Fig. 2e). The oocyte nucleus enlarged in size to $23\text{--}25\ \mu\text{m}$ in diameter at the late vitellogenic stages. Large quantities of granular ribosome precursors were present in the nucleoplasm and also as a layer of accumulated electron-dense material in the cytoplasm around the nuclear membrane (Fig. 2e).

The lyrate organ was composed of two cell types: the supporting cells and the nutritive cells, the latter forming a huge syncytium. The support-

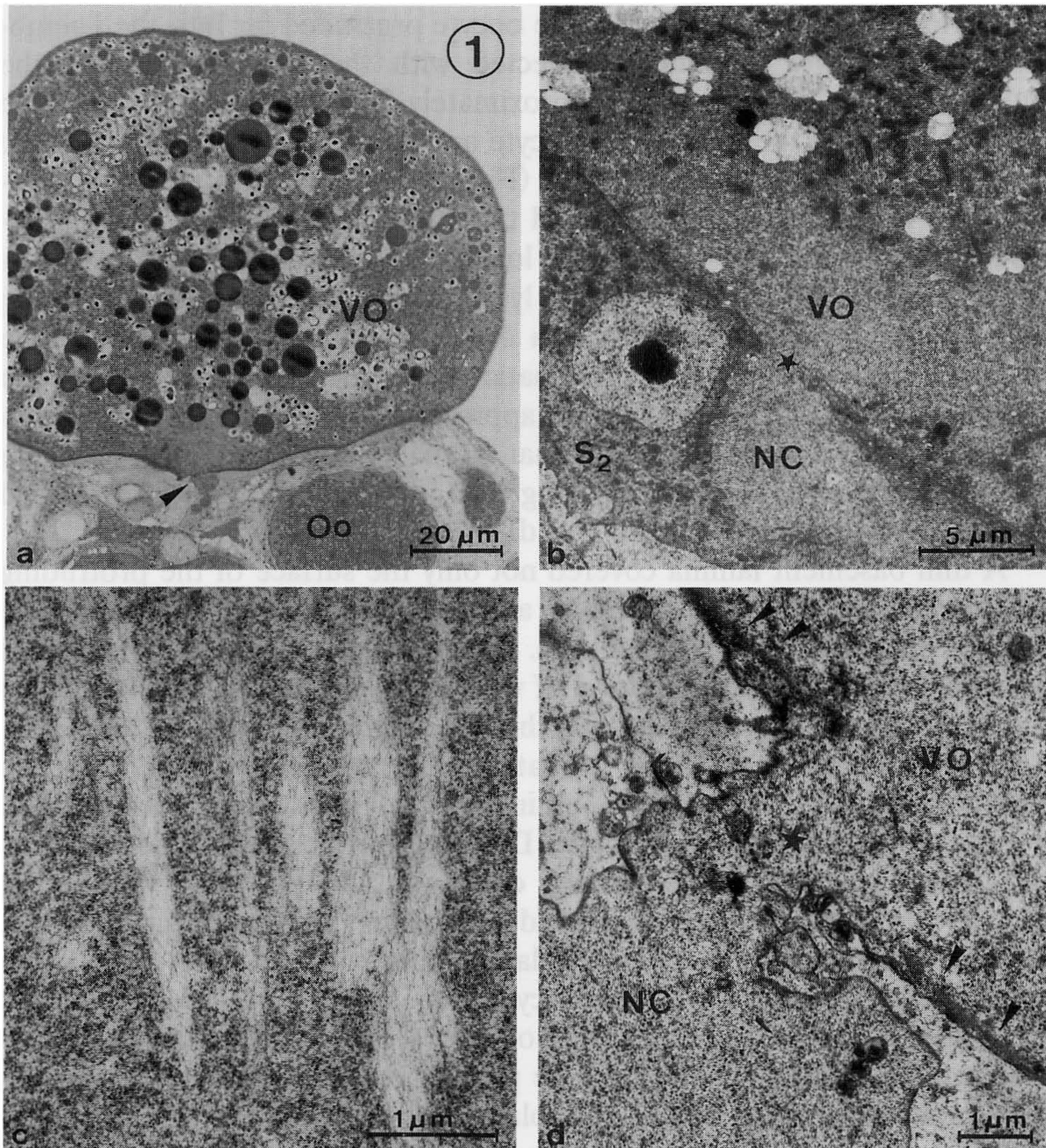


Fig. 1. Ovarian structures in *V. jacobsoni* during vitellogenesis. (a) Oocyte of stage 5 (VO) with dark yolk spheres and light areas containing glycogen and lipid droplets. In the part next to this large oocyte, young primary oocytes (Oo) and the insertion of the nutritive cord (arrow head) are seen. (b) Details of the insertion region (asterisk) of the nutritive cord (NC) surrounded by one of the two somatic cells (S₂) and part of the ooplasm (VO, vitellogenic oocyte stage 4) with numerous mitochondria and bright glycogen islands. (c) Part of the cytoplasm in the syncytial lyrate organ containing large numbers of dark ribosomes and showing light bundles of microfilaments directed towards the nutritive cord. (d) Stage 5 oocyte (VO) and connection (asterisk) to the nutritive cord (NC) with many ribosomes. The cytoplasm of the neighbouring somatic cells is almost free of ribosomes and, therefore, much less electron dense. Arrow heads point to the dense microfibrillar layer in the cortical ooplasm.

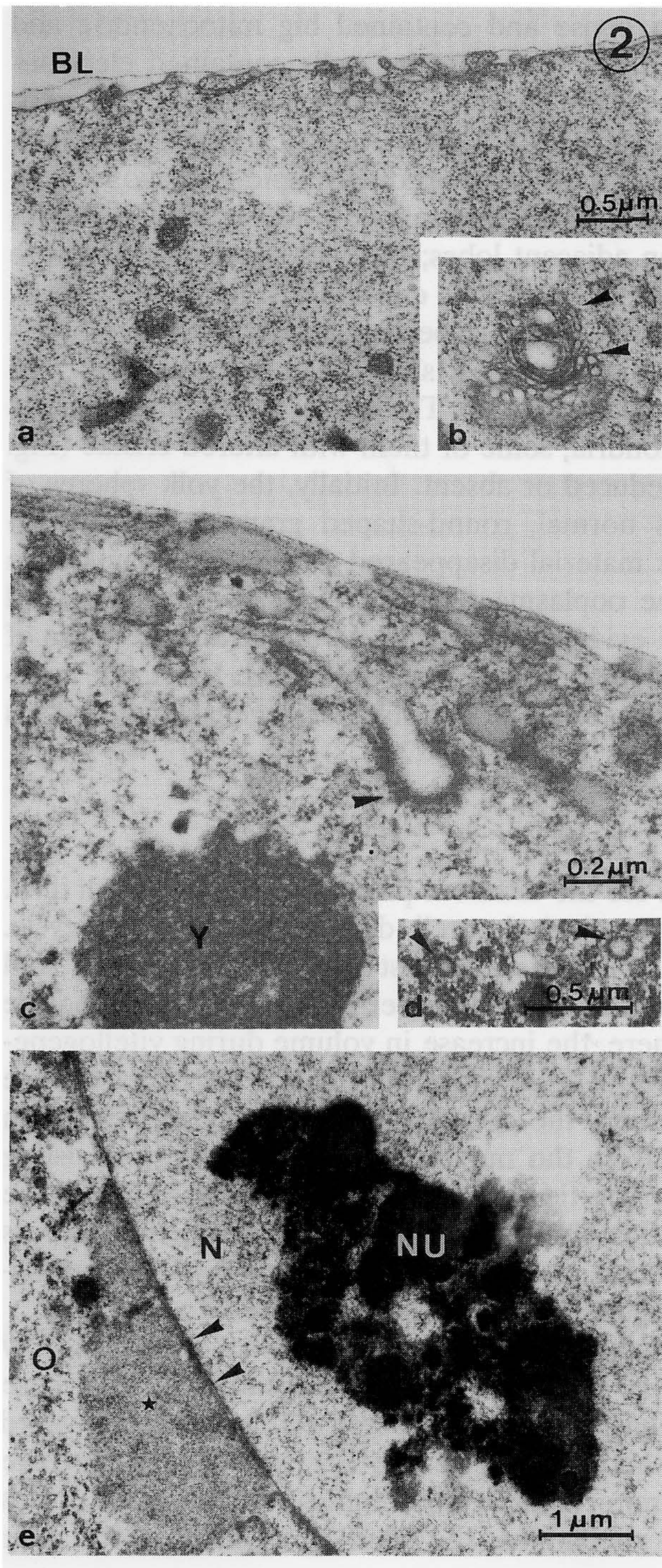


Fig. 2. Cortical and nuclear structures of *Varroa* vitellogenic oocytes of stages 4 and 5. (a) Cortical ooplasm with numerous ribosomes. The oocyte membrane forms microvilli and endocytotic structures; it is covered by a thin basement lamina (BL). (b) Ooplasmic Golgi apparatus. (c) Microvilli and clathrin-coated pits (arrow head), densely packed yolk platelet (Y). (d) Small clathrin coated vesicles (arrow heads) in the oocyte cortex. (e) Part of the germinal vesicle (N) with an enlarged nucleolus (NU). Electron dense material (asterisk) in the ooplasm (O) near the nuclear envelope (arrow heads).

ing cells were less electron dense and contained big mitochondria and residual bodies (Fig. 3a and 4a). The nutritive cells contained electron-dense cytoplasm because of the presence of large quantities of ribosomes. Some microfilaments (Fig. 1c), a little rough endoplasmic reticulum, many slender mitochondria and occasionally lipid inclusions were also seen. The huge nuclei with large nucleoli were highly lobulated with many contact zones between adjacent lobes; these bridge-like zones were formed by the two tightly apposed nuclear envelopes (Fig. 3b, 4b and c).

Some *Varroa* females, collected from hyperparasitized honey bee brood cells containing more than four females, showed an oocyte which presumably undergoes degeneration (Fig. 5a). The ooplasm included vacuoles and rounded, dense mitochondria, some of them with altered cristae (Fig. 5b). Glycogen areas were reduced or absent. Initially, the yolk spheres of these oocytes appeared as normal, round-shaped granules, but at advanced oosorption, the yolk material disappeared. Small cells of unknown origin seemed to invade the ooplasm. The microvilli were reduced and endocytotic activity was not evident any longer. At the advanced stages of oosorption, the ooplasm appeared rather disorganized. Even the mitochondria were barely recognizable. The final fate of degenerating oocytes is unknown.

DISCUSSION

The temporal requirements for the different phases of oocyte growth in *V. jacobsoni* can be studied particularly well during the first gonocycle. Pre-vitellogenic stages 1–3 of oogenesis last approximately 15 h and vitellogenic stages 4 and 5 another 15 h (Steiner *et al.*, 1994). If the oocyte is regarded as a regular sphere, the increase in volume during vitellogenesis is approximately 25 times in little more than half a day. We suppose that besides yolk incorporation, the oocyte is continuously supplied with cytoplasmic components from the nursing lyrate organ. The persisting nutritive cord enables this parasitic mite to complete oogenesis within the rather short time of approximately 30 h. Subsequent embryogenesis occurs within approximately 40 h. Thus, the initial oviposition is possible approximately 70 h after capping of the brood cell of the honey bee host.

The pre-vitellogenic growth phase in *V. jacobsoni* was subdivided by Alberti and Zeck-Kapp (1986) into three stages of oogenesis. During these stages, mitochondria and ribosomes were observed within the trophic cord between the lyrate organ and the oocyte. As a result of this nutritive supply, large numbers of mitochondria accumulated in the ooplasm, as demonstrated by selective rhodamine 123 staining (Steiner, 1992). Our

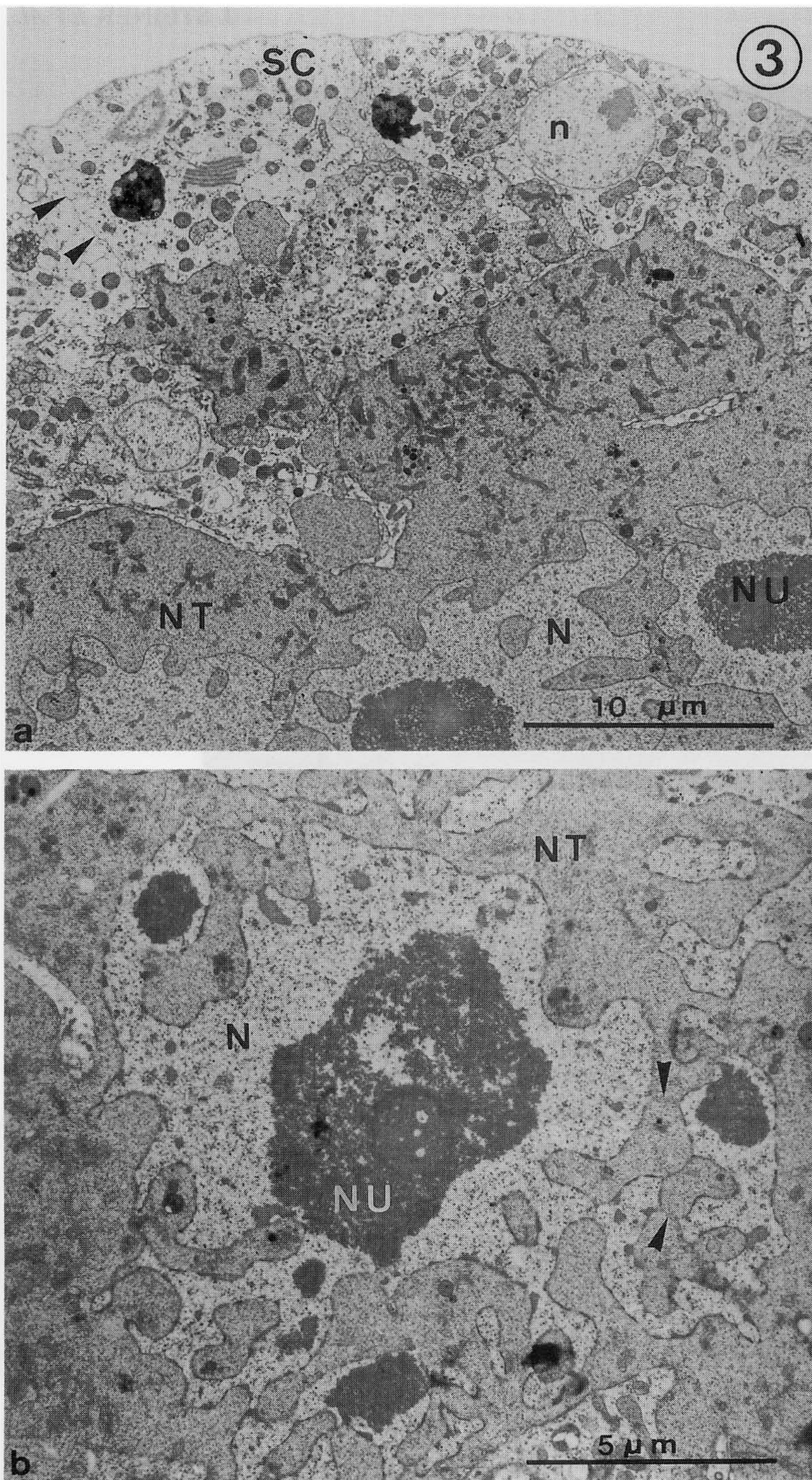


Fig. 3. Lyrate organ during (a) stage 4 and (b) stage 5 of *Varroa* oogenesis. The marginal supporting cells (SC) are easily recognized by their electron-light cytoplasm and small round nuclei (n). Cell boundaries are indicated by arrow heads. The syncytial nutritive tissue (NT) is characterized by rather electron-dense cytoplasm, huge lobulated nuclei (N) and large nucleoli (NU). Arrow heads point to contact zones between adjacent nuclear projections.

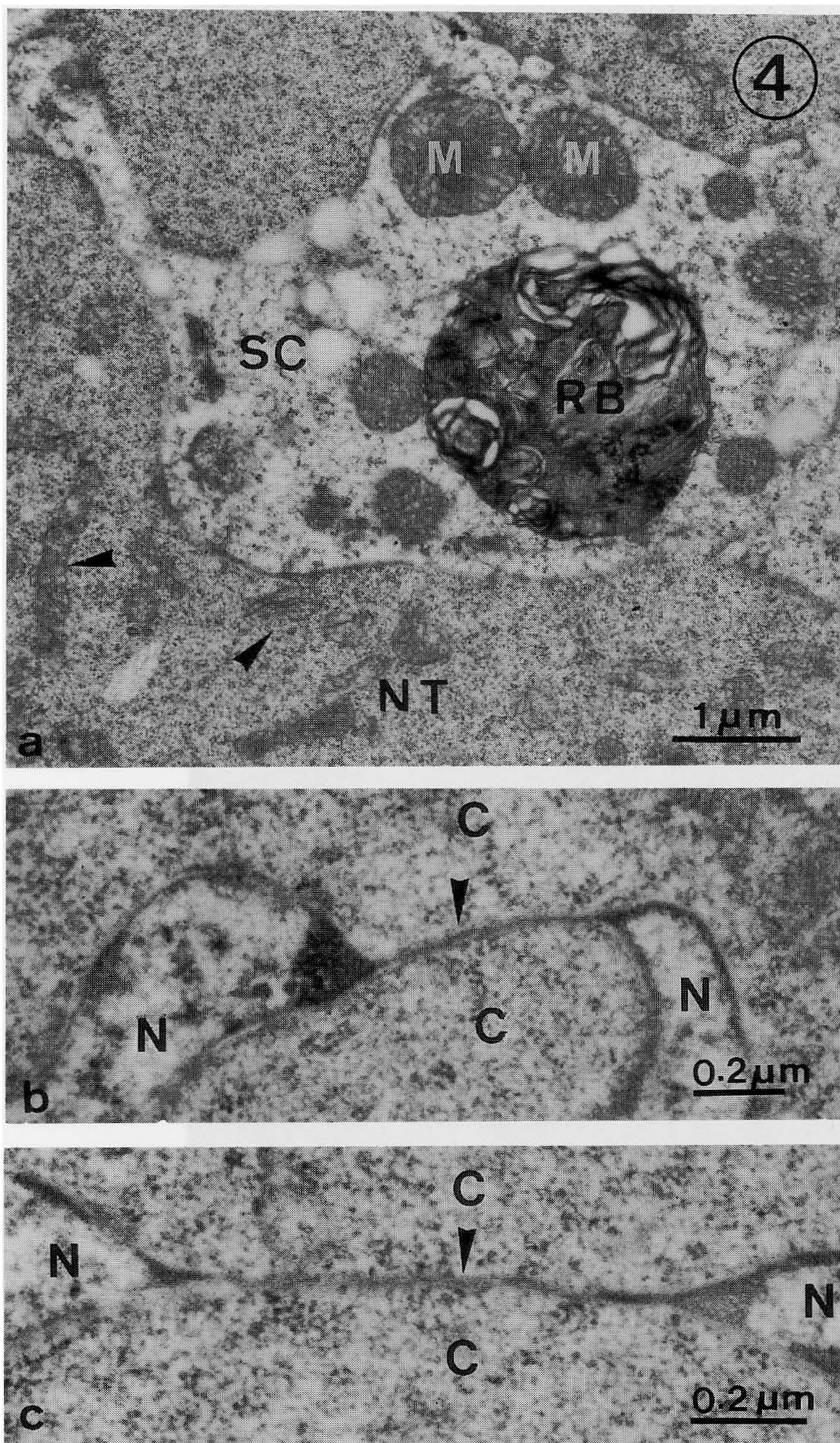


Fig. 4. Detailed structures of the lyrate organ during stage 4 of *Varroa* oogenesis.

Fig. 5. Oocyte degeneration in *V. jacobsoni* living under stress conditions in host brood cells hyperparasitized by more than four females. (a) Ooplasm (O) with a large vacuole (v) close to the germinal vesicle (n). Vanished yolk and glycogen stores indicate oosorption. LO = lyrate organ. (b) Degenerating mitochondria showing unusual cristae structures (arrow heads).

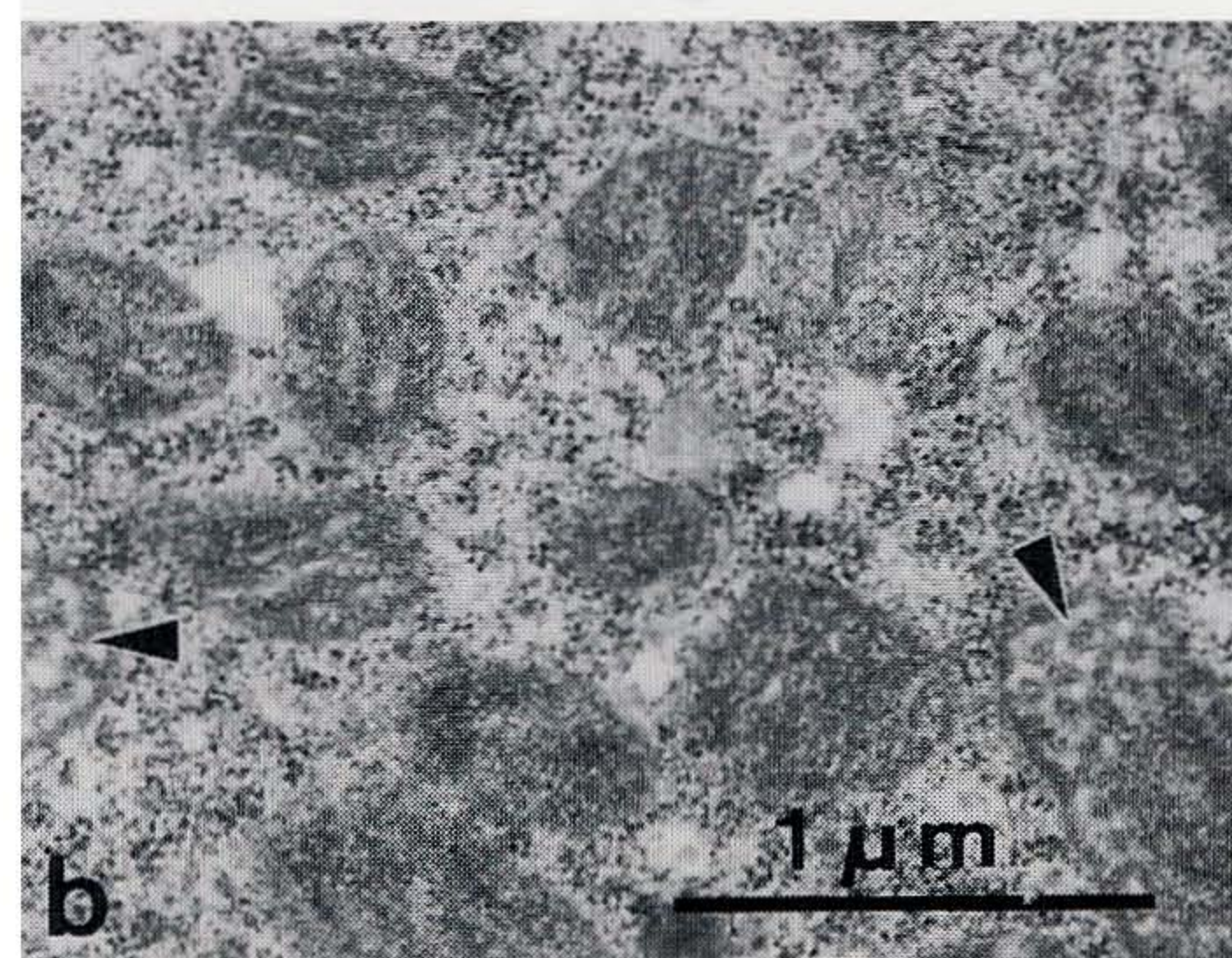
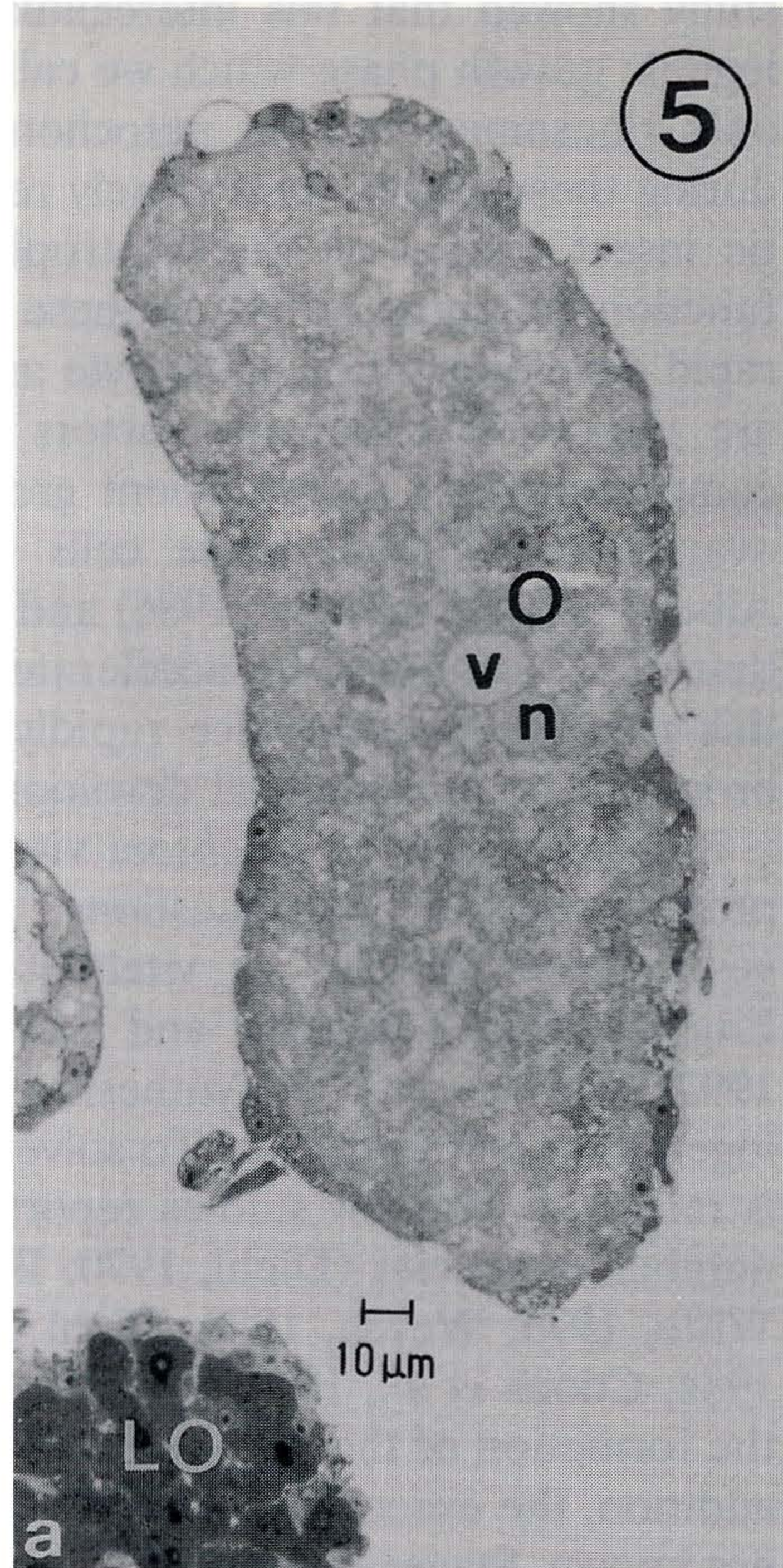


Fig. 4. (Facing page, p. 418) Detailed structures of the lyrate organ during stage 4 of *Varroa* oogenesis. (a) Part of a supporting cell (SC) with residual bodies (RB) and large mitochondria (M), surrounded by syncytial nutritive tissue (NT) containing much smaller, slender mitochondria (arrow heads) and numerous ribosomes. (b and c) Contact zones (arrow heads) between adjacent nuclear lobes (N) consisting of the two tightly apposed nuclear envelopes. C = cytoplasm.

study showed that this intercellular connection persisted into the vitellogenic growth phase which we call stages 4 and 5 of oogenesis. However, only ribosomes, but no mitochondria, were seen in the nurse strands during these stages. This closely resembles the situation found in meroistic insect ovaries of the telotrophic type of ovarioles in structure and function (Bünning, 1994). In these modern meroistic insects, the resulting rapid development of the oocyte and the short duration of embryogenesis are important enhancing factors for successful radiating evolution, in comparison with more ancient groups of insects equipped with panoistic ovaries and without nurse cells (Bier, 1969). As already proposed by Alberti and Zeck-Kapp (1986) and Alberti and Hänel (1986), we conclude that in *V. jacobsoni* the accelerated oogenesis and embryogenesis enable this parasite to reproduce rapidly during the short span of the capped brood in the pre-imaginal development of the honey bee.

Many details in *V. jacobsoni* vitellogenesis such as the origin of the yolk proteins remain open questions. Immunological evidence of using undigested host proteins as vitellogenins (Tewarson, 1982; Tewarson and Engels 1982; Tewarson and Jany, 1982) is accumulating (Michelette, 1992), but the site of synthesis of additional endogenous *Varroa* vitellogenins is still unknown. To solve this problem, more experimental work is required. The few studies reported for other acari cover only a limited number of species (Diehl, 1970; Brinton and Oliver, 1971; Alberti, 1974; Witte, 1975; Weyda, 1980; Diehl *et al.*, 1982; Mothes-Wagner and Seitz, 1984; Coons *et al.*, 1986; Witaliński, 1993). Other open questions include the formation of the egg shell and the cells contributing to this activity. In addition, the exact time of the fertilization process and its localization are not known. Stages 4 and 5 of *Varroa* oogenesis do not seem to include this event.

Besides normal growth and differentiation of the *Varroa* oocyte, we studied some aspects of oosorption. Egg degeneration is commonly known to occur in arthropods. In the mite *C. mycophagus* unfertilized eggs are incapable of parthenogenetic development and degenerate (Heinemann and Hughes, 1970). Here, the oocyte migrates into the oviduct, but no chorion is formed. Similarly, in fed virgin argasid ticks, egg resorption is induced (Diehl *et al.*, 1982). For the first time, observations on oosorption in *V. jacobsoni* indicate that stress as caused by severe reproductive competition (Donzé, 1995) also may lead to oosorption. This phenomenon needs to be studied in more detail in the future.

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