

Ultrastructural and histological changes induced by ivermectin in the ovary of *Argas persicus* after feeding

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Abstract

The ovarian wall of *A.persicus* consists of primary oocytes of three developmental stages namely , young , previtellogenic and vitellogenic in addition to interstitial cells . After feeding and mating , the three stages and interstitial cells , particularly funicle cells that carry oocytes , markedly increased in size and their cytoplasmic organelles exhibit notable changes correlated with yolk and egg shell formation .

The present study examined the histological and ultrastructural aspects during the formation of yolk and egg shell. The first seem to originate from small vesicles derived from Golgi bodies, rough endoplasmic reticulum and mitochondrial cristae. The egg shell seems to develop from vesicles derived from Golgi bodies that fuse with the cell membrane and discharge their content into the extracellular space between basement membrane and tunica propria. Glycogen aggregates and lipid droplets are commonly observed between yolk spheres.

The injection of selected dose of ivermectin (400 $\mu\text{g}/\text{kg}$) prevented growth and development of oocytes. The ovary appeared studded with young and previtellogenic primary oocytes surrounding a narrow ovarian lumen or the ovarian wall, carrying oocytes, is stretched around a large fluid filled lumen. Surface microvilli of primary oocytes and coated vesicles underlying them became comparatively fewer. Mitochondria and rough endoplasmic reticulum became fewer, fragmented and finally degenerated in primary oocytes and funicle cells. Ivermectin interfered with the formation of yolk granules and egg shell. However, in the few formed vitellogenic oocytes, Golgi bodies were not affected. The egg shell appeared thinner with low electron density. The cytoplasm of funicle cells became highly vacuolated, its organelles, hardly distinguished and nuclei became swollen without definite nuclear membrane. Ivermectin completely prevented the formation of glycogen particles. Our results suggests that ivermectin causes partial blockage of the release of the vitellogenetic hormone that regulates vitellogenesis and other aspects of oogenesis as indicated by disrupted synthesis and uptake of yolk protein in the developing oocytes.

Introduction

The fowl tick *Argas (Persicargas) persicus* is a specific parasite of domestic and certain wild birds in parts of Asia, Europe, Africa and Australia (Nosek et al.,1980). The *Argas* toxins affect afferent and efferent nerve fibres and influence neuromuscular transmi -

ssion and thus cause fowl paralysis which increases in approximately direct proportion to the degree of tick infestation (Gothe et al.,1979). Numerous if not all argasid species produce more or less severe irritation when biting man (Yu Quan et al.,1995).

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A.persicus may occasionally bite man and transmit *Bacillus anthracis* (anthrax) to man (Deputy and Kaweh, 1937). Some *Argas* species infesting domestic birds were recorded to attack people causing severe pruritus, acute urticaria, inflammation with erythema, discoloration, bruise-like lesions, ulcerations, fever, weakness and anaphylactic shock (Genchi, 1992; Veraldi et al., 1998).

The macrocyclic lactone ivermectin derived from the soil actinomycete *Streptomyces avermitilis* was introduced to the marketplace as an antiparasitic drug in 1981 (Campbell, 1989). It is now commercially used for the treatment and control of parasites in many domestic and laboratory animals and in man (Einstein et al., 1994). The studies on ivermectin were mainly confined to its effect on parasites, on biological parameters and routes of administration (Lancaster et al., 1982; Kaufman et al., 1986; Mahmood et al., 1991). However, rare data dealt with the histological, histochemical or ultrastructural changes occurring in the internal organs of these parasites. The present study was undertaken to assess the effect induced by this drug on the internal organs of the tick *A.persicus* after feeding on hosts injected by the selected effective dose, which may provide an explanation for the mechanism of action of this drug.

Materials And Methods

Argas (Persicargas) persicus used in the present work originated from ticks obtained from a fowl house in Giza Governorate in Egypt. The domestic pigeon *Columba livia domestica* was used as a host. The ticks were reared in polyethylene tubes in an incubator at $28 \pm 1^\circ\text{C}$ and relative humidity of 75%. Pigeons were treated by a single subcutaneous

injection in the femur region with Ivomec Injectable[®] (a formulation containing ivermectin in 1% concentration, MSD Agvet, Division of Merck and Company, Rahway, N.J.). The effective dose was selected according to the results obtained from an investigative study on some biological parameters such as viability, sexual behavior, amounts of ingested blood, emitted coxal fluid, blood digestion and fertility (Montasser, 2000). The used dose rate was 4000 $\mu\text{g}/\text{kg}$ pigeon weight versus control. Injections were made 2 and 7 days before ticks were allowed to feed. Tick feeding was also carried out 2 and 3 weeks after injection with the selected effective dose. Ovaries of the unfed females and fed females at 0, 2, 4 and 6 days after feeding were investigated. They were dissected in 0.7% NaCl solution, the dorsal integument was removed and internal organs were quickly fixed in Bouin's or calcium formol's fixatives, dehydrated in ethyl alcohol and then transferred to methyl benzoate for 24 hours to soften the tegument. They were then transferred to a solution of 2% celloidin in methyl benzoate for another 24 hours, cleared in benzol for 2 minutes, infiltrated and embedded in paraplast. Serial sections were cut at 5 μm thick and stained with Mallory triple (MT) and haematoxylin and eosin (HX&E) stains for general observations.

For transmission electron microscopy, female ticks were dissected out under cold phosphate buffer adjusted to the pH 7.4 and fixed for 2 hrs in cold fresh buffered glutaraldehyde. Organs were washed in phosphate buffer, postfixed in cold buffered 1% osmium tetroxide for 1 hr and washed again in fresh buffer for 0.5 hr. They were then dehydrated in ethanol, transferred through acetone for 0.5 hr, acetone-epoxy resin solution (1:1) for 1 hr,

epoxy resin at room temperature for 2hrs and embedded in epoxy resin. Semi-thin sections (1 μm) were stained with methylene blue stain (MB) while ultrathin sections were stained in 2% uranyl acetate and lead citrate, examined and photographed using a Phillips transmission electron microscope (Phillips Co., Holland).

Results

The ovary in *A. persicus* is a hollow broad strap-like tube that lies transversely across the posterior half of the body.

The ovarian lumen is surrounded by the ovarian wall consisting of interstitial cells and primary oocytes in various stages of development (Figs. 1 - 21). The ovarian wall is covered externally by a thin layer tunica propria (ca. 0.3 μm thick) that consists of finely fibrillar material (Fig. 9a), separating the ovarian tissue from the haemolymph. After feeding and mating, oocytes protrude into the haemocoel giving the ovary a grape-like appearance (Figs. 2 and 3).

Oocyte development in the present study is observed in unfed females and daily 6 days after feeding and mating. Histological examination reveals three developmental stages of primary oocytes namely, young oocytes, previtellogenic oocytes and vitellogenic oocytes during this period. Oocytes of unfed ticks are oval, elongated or polygonal and are found facing the ovarian lumen (Figs. 1 and 6). From EM examination, their dense cytoplasm is found to contain few small ovoid or round mitochondria, free ribosomes and polyribosomes, very few cisternae of rough endoplasmic reticulum in addition to numerous solitary and colonial forms of rickettsia-like microorganisms which are usually found infecting the different types of

ovarian cells (Fig. 6). The nuclei are large with irregular or rounded margins (Fig.6). The nucleoplasm is occupied with finely granular euchromatin and dense masses of heterochromatin lying beneath the nuclear membrane in addition to a comparatively large nucleolus (ca. 4 μm diameter). After feeding, young oocytes measurements range between $15.4 \pm 0.8 \times 12.6 \pm 0.7 \mu\text{m}$ and $22.1 \pm 0.99 \times 16.6 \pm 0.7 \mu\text{m}$ (Table 1). Diameter of their nuclei also ranging between $8.3 \pm 0.4 \mu\text{m}$ and $11.7 \pm 0.48 \mu\text{m}$ (Table 1). No marked structural changes are observed directly after feeding except that the mitochondria become numerous and fill most of the oocyte cytoplasm (Fig. 7).

Phase 2 or the previtellogenic oocyte development is initiated by feeding and mating and is marked by a great cytoplasmic growth and lasts until the appearance of the vitelline granules (Figs.1-3). The mean diameter of oocytes in this stage ranges between $69.2 \pm 3.7 \times 53.7 \pm 3.1 \mu\text{m}$ and $85.3 \pm 3.2 \times 61.8 \pm 2.99 \mu\text{m}$ during the examined period (Table 1). In the unfed ticks, the cytoplasm is dense, granulated and contains numerous vacuoles, small mitochondria and numerous aggregates of rickettsia-like microorganisms (Figs.8a,b). Below the tunica propria, the surface of the oocyte membrane shows few microvilli (Fig. 9a) that become numerous and long after feeding (Figs.9b and 13). After feeding, several pits and coated vesicles that were formed due to the process of micropinocytosis are found in the peripheral zone of the oocyte cytoplasm (Figs.9b and 13). Microtubules are commonly observed as well as large number of microvilli and coated vesicles (Fig. 13). Cytoplasmic organelles become well developed within the few days after feeding. Mitochondria increase in their number

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(Fig. 10) and rough endoplasmic reticulum fills the cytoplasm and their cisternae appear as swollen vacuoles of short lengths (Figs. 13 and 14a). The Golgi complex markedly develops and increases in number (Fig.14a). Lipid droplets appear as groups scattered in the cytoplasm (Fig. 14b). The nucleus of the previtellogenic primary oocytes extremely enlarges within the few days following feeding (Fig.12). Its mean diameter ranges between $26.2 \pm 1.4 \mu\text{m}$ and $33.1 \pm 0.97 \mu\text{m}$ (Table 1). The nucleoplasm is totally occupied with fine granules of euchromatin and a prominently large nucleolus. Numerous perichromatin granules are aggregated in the nucleoplasm particularly beneath the nuclear membrane. Similar granules are also noticed outside the nuclear membrane (Fig. 11). The vitellogenic primary oocytes of *A. persicus* begin with the appearance of the first yolk granules in the cytoplasm and end with ovulation (Figs. 4,5 and 15-18). Oocytes in this growth phase are characterized by the formation of yolk granules in addition to the deposition of egg shell. Yolk granules appear by the 4th day after feeding (Fig.4). In this phase oocytes measure $170.6 \pm 12.4 \times 117.9 \pm 8.77 \mu\text{m}$ 4 days after feeding and $238.5 \pm 21.1 \times 159.5 \pm 12.7 \mu\text{m}$ 6 days after feeding (Table 1). The nucleus at the early stages of yolk deposition is obvious (Fig. 4), but in later stages it becomes diffused and difficult to detect among yolk bodies. Mean diameter of the nucleus in this phase ranges between $43.5 \pm 1.39 \mu\text{m}$ and $47.4 \pm 1.33 \mu\text{m}$ (Table 1). Yolk seems to originate from the small vesicles derived from Golgi bodies and rough endoplasmic reticulum (Figs. 16). Some coated vesicles that come from micropinocytotic pits are seen fused into the multivesiculate bodies (Fig. 15). Multivesicular bodies gradually loss

their internal membranes and eventually form large membrane limited reservoirs filled with fine granules (Fig. 16). Through repeated fusions between these reservoirs as well as small vesicles, derived from Golgi bodies and endoplasmic reticulum, large electron dense homogeneous yolk spheres are formed and gradually fill most of the oocyte cytoplasm (Fig. 18). By the 6th day after feeding, i.e. in more advanced oocytes, there are numerous small and large vacuoles (Figs. 17 and 18). Mitochondria become fewer in number with less electron density and thinner cristae (Figs. 17 and 18). On the other hand glycogen particles increase and enormously aggregate in large rosette-like deposits that fill most of the cytoplasm between the yolk granules and lipid droplets increase in size and quantity as well (Figs.17 and 18).

Mature oocytes (eggs) in the ovarian wall appear to be covered with shells (Fig. 5). Deposition of egg shell begins slightly later to the beginning of yolk formation (Fig. 17). Egg shell is synthesized mainly by the oocyte itself. It seems to develop from vesicles, derived from Golgi bodies, that pass towards and fuse with the cell membrane and/or microvilli discharging their content into the extracellular space between the basement membrane and tunica propria (Fig. 17). Released material are probably polymerized into dark patches that are firstly added near the surface of the oocyte then directed to the outside (Fig. 17). By the 6th day of feeding, the mean thickness of the egg shell reaches ca. $2.2\text{-}2.8 \mu\text{m}$. Gradual increase in egg shell deposition results in stretching of the tunica propria giving the appearance of fewer distinct fibres (Fig. 17). During shell deposition microvilli reach their maximum length and appear as straight tubes (Fig. 17).

The interstitial cells are small, irregular in shape and rest on the ovarian membrane between primary oocytes (Fig. 6). Their cytoplasm is extensively occupied by rickettsia-like microorganisms, few small mitochondria and vacuoles in addition to large oval or polygonal nuclei occupy most of the cell volume (Figs. 6 and 7). Their nuclei appear filled with euchromatin and heterochromatin as well.

The funicle cells carrying the previtellogenic oocytes in unfed females are irregular in shape (Fig.19) and collectively coated with the tunica propria covering the oocytes (Figs.20 and 21). They possess large nuclei that are occupied with fine chromatin granules and few chromatin aggregations mainly underlying the nuclear membrane (Fig.19). Their cytoplasm contains only few cisternae of rough endoplasmic reticulum, small mitochondria and little of the polyribosomes and microtubules. Funicle cells are fastened to each other by several desmosomes (Fig. 20). After feeding, their walls become highly convoluted (Fig. 21). Apical surfaces have numerous invaginations that are deeply inserted between oocyte microvilli (Fig. 20). After feeding, numerous microvilli are observed in the area connecting the oocyte with follicle cells and the cytoplasm appears highly occupied with numerous short cisternae of rough endoplasmic reticulum, several elongated mitochondria, few Golgi bodies and lysosomes (Figs. 20 and 21). Cytoplasmic areas near the connection with oocytes contain few of these organelles and numerous microtubules (Fig. 20). The nuclei contain numerous patches of heterochromatin lying mainly under the nuclear membrane (Fig. 21).

Effect of ivermectin on the structure of the developing oocytes:

Considerable effects are noticed in the ovary of *A. persicus* following administration of a single subcutaneous dose of ivermectin (400 $\mu\text{g}/\text{kg}$) given to the host pigeon (Figs. 22-33). It mostly prevents growth and development of oocytes. The ovary appears studded with young phases of primary oocytes (Fig. 22). Previtellogenic phases are mainly from earlier development in the last nymphal instars. The ovarian lumen is filled with a fluid making the ovary more dilated and the ovarian wall stretched (Fig. 24). The sizes of oocytes, particularly young ones, and their nuclei were larger in ticks fed on untreated hosts than those fed on treated ones. Dimensions of young oocytes 4 and 6 days after feeding are significantly higher ($p < 0.01$) in females fed on untreated hosts when compared to those fed on treated ones. The latter measuring $14.1 \pm 0.4 \times 11.5 \pm 0.4 \mu\text{m}$ and $14.6 \pm 0.6 \times 10.1 \pm 0.4 \mu\text{m}$, respectively (Table 1). Mean diameter of nuclei 2, 4 and 6 days after feeding are significantly higher ($p < 0.01$) in females fed on untreated hosts than on treated ones measuring $9.32 \pm 0.3 \mu\text{m}$, $8.54 \pm 0.23 \mu\text{m}$ and $8.8 \pm 0.17 \mu\text{m}$, respectively (Table 1). No obvious changes are observed in staining affinity with MT or methylene blue stains in young oocytes or their nuclei between ticks fed on untreated and treated hosts (Figs. 22 and 23). Electron micrographs show that the treatment causes disruption in some areas of the plasma membranes in addition to slight vacuolation in the cytoplasm (Figs. 26). Mitochondria are clearly noticed as in oocytes of ticks fed on untreated hosts but other organelles are rarely observed (Figs. 26).

Dimension of the previtellogenic phases of primary oocytes and their nuclei of ticks fed on untreated and treated hosts shows no significant difference ($p > 0.05$) (Table 1). No

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marked changes are noticed in MT or methylene blue staining affinity of the previtellogenic phases and their nuclei between ticks fed on untreated and treated hosts (Figs. 22-25). However electron microscope reveals that the previtellogenic oocytes of ticks fed on treated hosts exhibit considerable alterations from those fed on untreated ones (Figs. 28-29b). Microvilli surrounding oocytes are also found as in the untreated group but with less density (Figs. 17 and 27). Coated and micropinocytotic vesicles below these microvilli are comparatively fewer. The cytoplasm appears less dense with fewer organelles. Mitochondria become fewer and smaller (Figs. 28 and 29b). The cristae of some mitochondria are fragmented, degenerated so the mitochondria look like round or oval vesicles containing dark granules and surrounded with double membranes (Fig. 29b). Others are completely degenerated leaving only masses of granulated substance without limiting membrane. Some cytoplasmic areas particularly near funicle cells appear vacuolized (Fig. 28). The nuclei of oocytes at this stage did not show considerable changes as compared with those of untreated group besides the perichromatin granules that are released from oocyte nucleus are considerably fewer (Fig. 28).

Ivermectin greatly affects vitellogenesis that only appears in less than 1/10 of the examined specimens, so it is difficult to statistically measure this kind of treated oocytes (Table 1). The cytoplasm appears loose and less electron dense than in ticks fed on untreated hosts. Most of the mitochondria are destructed, their cristae change into fine granular material and their matrices appear less dense (Figs. 30 and 31). Rough endop-

lasmic reticulum become fewer giving the appearance of short vesicles with little ribosomes. Development of Golgi bodies is nearly unaffected by ivermectin treatment. Vesicles of Golgi bodies, short cisternae of rough endoplasmic reticulum and degenerated mitochondria still seem to share in yolk formation. By the 6th day after feeding few membrane limited yolk spheres containing fine and less electron dense granules are formed. Fewer lipid droplets are observed in vitellogenic oocytes of ticks fed on treated hosts (Figs. 30 and 31). Ivermectin completely prevent formation of glycogen granules that are commonly observed as large rosette-like deposits in oocytes of ticks fed on untreated hosts.

Ivermectin considerably affect shell deposition, where only a few coated vesicles containing moderately dense material are noticed near the oocyte membrane. They seem to discharge their contents through exocytosis to the outside between microvilli and tunica propria. Microvilli are not stretched as in oocytes of ticks fed on untreated hosts. Egg shell appears thinner where its thickness measures about half of the oocyte of ticks fed on untreated hosts (Figs. 17 and 31).

Impact of ivermectin on funicle cells is also drastic (Fig. 32 and 33). They become intensively vacuolized, hypertrophied and greatly damaged. Cytoplasmic organelles become fragmented and could be hardly distinguished. Nuclei appear as swollen irregular masses without definite nuclear membranes (Figs. 32 and 33). Invaginations and desmosomes normally found near the oocyte-funicle cells attachment site are very few as compared with the condition of ticks fed on untreated hosts (Fig. 20 and 33).

Table 1: Dimensions of the oocytes and their nuclei at differend developmental stages of *Argas (P.) persicus* fed on untreated and ivermectin treated hosts with a single dose of subcutaneous injection of 400 ug/kg 0,2,4 and 6 days after feeding .

Time after Feeding	Mean oocyte dimension (μm) \pm SE (Range)		Mean nucleus diameter (μm) \pm SE (Range)	
	Untreated	Treated	Untreated	Treated
Zero Day				
Young Oocyte	15.4 \pm 0.8 x 12.6 \pm 0.7 (10.5-24.0 x 9.0-24.0)	14.3 \pm 0.3 x 12.2 \pm 0.3 (10.5-18 x9-15)	8.3 \pm 0.4 (6-12)	8.14 \pm 0.25 (6.6-9)
Previtellogenic Phase	69.2 \pm 3.7 x 53.7 \pm 3.1 (45-111 x 30-105)	69.0 \pm 3.7 x 49.7 \pm 3.0 (33-108 x 24-78)	26.2 \pm 1.4 (15-42)	23.0 \pm 0.92* (12-33)
Vitellogenic Phase	-	-	-	-
Two Days				
Young Oocyte	18.4 \pm 0.7 x 14.5 \pm 0.5 (12-22.5 x 12-18)	15.3 \pm 0.5 x 12.8 \pm 0.5 (12-19.5 x 10.5-16.5)	11.17 \pm 0.35 (9-13.5)	9.32 \pm 0.30** (6.9-12)
Previtellogenic Phase	69.9 \pm 3.5 x 54.1 \pm 2.8 (42-105 x 33-78)	67.1 \pm 2.5 x 55.9 \pm 2.0 (45-102 x 36-75)	26.7 \pm 0.87 (18-33)	29.1 \pm 1.28 (21-45)
Vitellogenic Phase	-	-	-	-
Four Days				
Young Oocyte	26.2 \pm 1.2x20.6 \pm 1.04 (18-34x12-33)	>14.1 \pm 0.4x11.5 \pm 0.4 ** (10.5-18 x 10.5 – 18)	13.3 \pm 0.58 (7.5-16.5)	8.54 \pm 0.23 ** (7.5-10.5)
Previtellogenic Phase	83.3 \pm 3.18x58.4 \pm 2.4 (57-117x36-78)	86.1 \pm 3.9 x 62.2 \pm 3.5 (57-120 x 39-99)	31.8 \pm 0.73 (27-39)	30.4 \pm 1.18 (24-39)
Vitellogenic Phase	170.6 \pm 12.4x117.9 \pm 8.77 (102-276x75-240)	-	43.5 \pm 1.39 (36-57)	-
Six Days				
Young Oocyte	22.1 \pm 0.99x 16.6 \pm 0.7 (18-33 x 12-24)	>14.6 \pm 0.6x 10.1 \pm 0.4** (12-18 x 9.12)	11.7 \pm 0.48 (9-16.5)	8.8 \pm 0.17** (8.4-9.6)
Previtellogenic Phase	85.3 \pm 3.2x61.8 \pm 2.99 (60-120x42-84)	81.0 \pm 4.03 x 53.7 \pm 2.57 (48-108 x 39-75)	33.1 \pm 0.97 (30-42)	31.5 \pm 1.28 (22.5-42)
Vitellogenic Phase	238.5 \pm 21.1x159.5 \pm 12.7 (111-432x87-300)	-	47.4 \pm 1.33 (38-60)	-

* Significant difference (P < 0.05), ** Highly significant difference (P<0.01)

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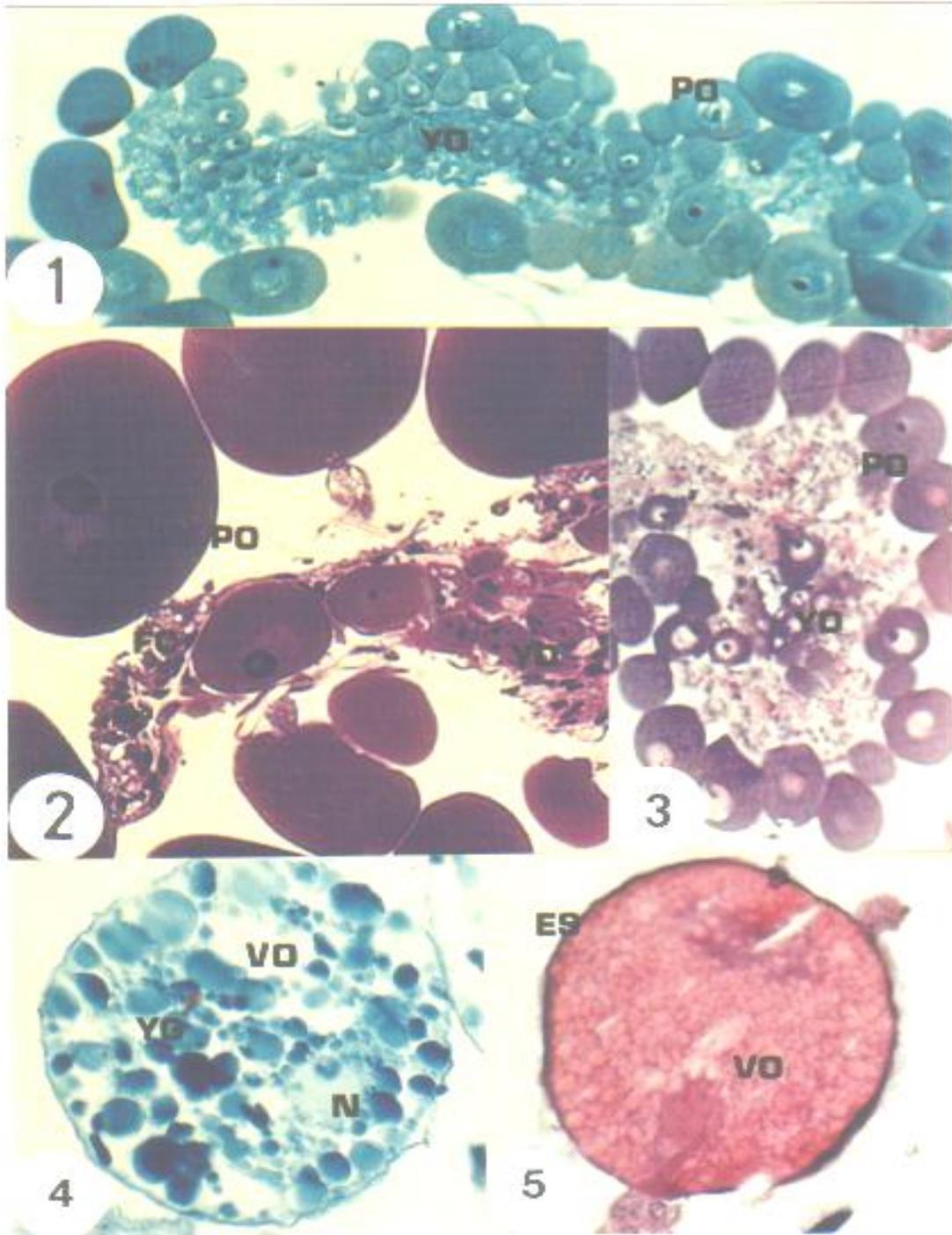


Fig. 1: Transverse section passing through the ovary of unfed *A. persicus* showing young (YO) and previtellogenic (PO) stages of primary oocytes (MT stain, x 160).

Fig. 2: Semithin section of the ovary of *A. persicus* directly after feeding showing young (YO) and previtellogenic (PO) stages of primary oocytes. FC, funicle cells (MB stain, x 200).

Fig. 3: As in Fig. 1 but 2 days after feeding (Hx-E stain, x 125).

Fig. 4: Transverse section passing through vitellogenic oocyte (VO) in the ovary of *A. persicus* 4 days after feeding showing the formation of yolk granules (YG), N, nucleus (MT stain, x 200).

Fig. 5: As in Fig.4 but 6 days after feeding showing the formation of egg shell (ES) (Hx-E stain, x 200).

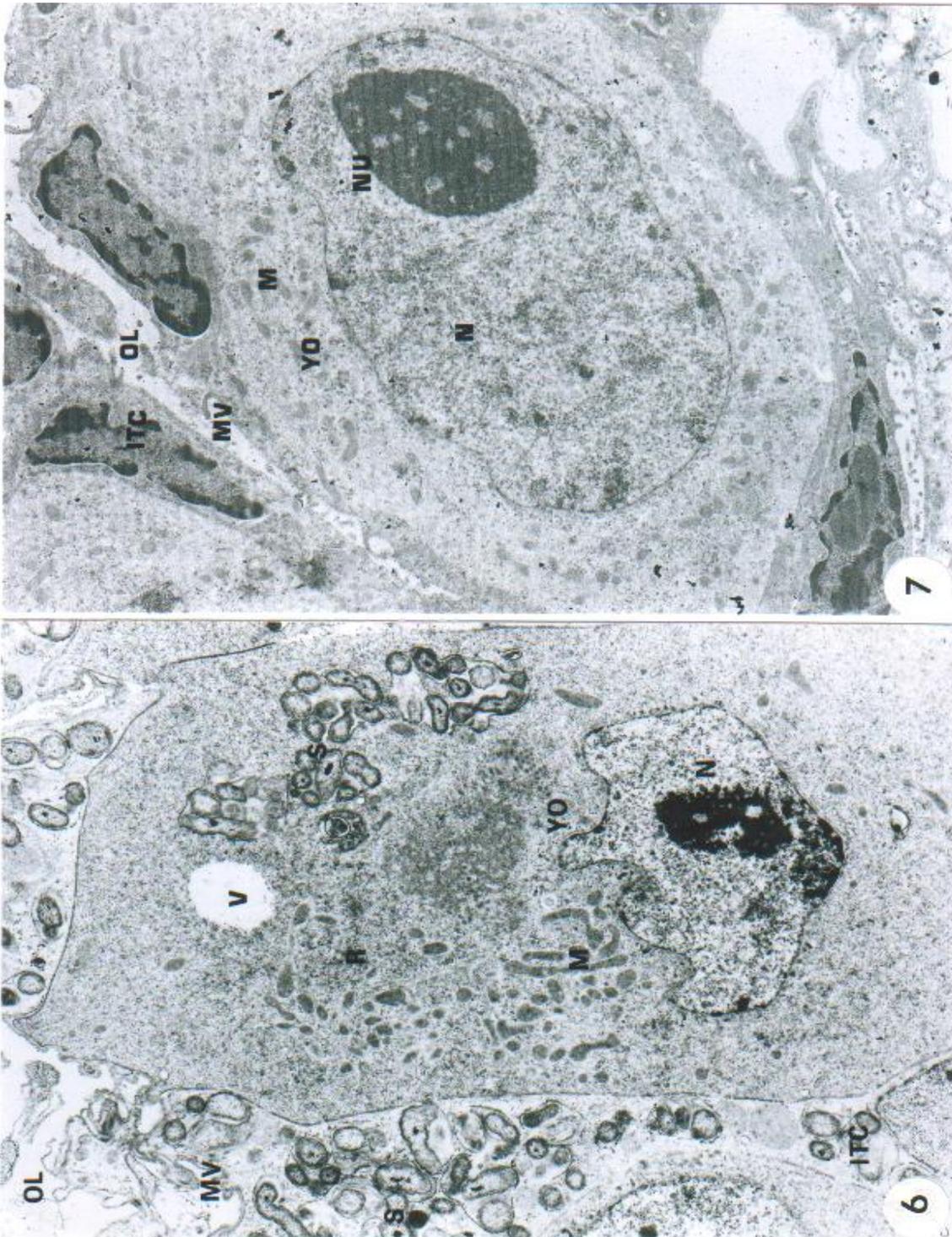


Fig. 6: Electron micrograph of young oocyte (YO) of unfed *A. persicus*. ITC, interstitial cells; M, mitochondria; MV, microvilli; N, nucleus; OL, ovarian lumen; R, ribosomes; S, rickettsia-like microorganisms; V, vacuole (x 9750).

Fig. 7: Electron micrograph of young oocyte (YO) of *A. persicus* directly after feeding showing mitochondria (M), nucleus (N), nucleolus (NU), ovarian lumen (OL), microvilli (MV) and interstitial cells (ITC) (x 13000).

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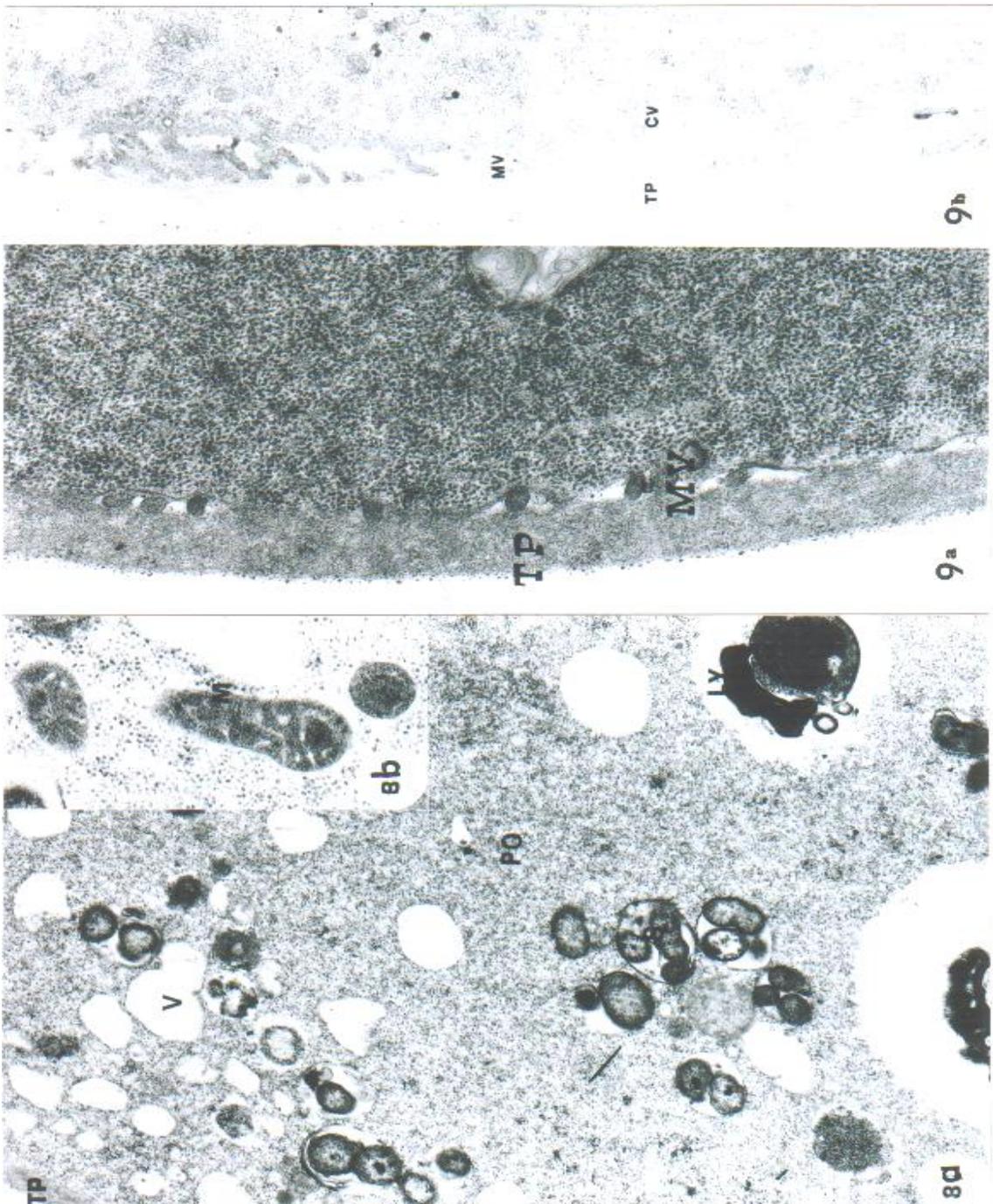


Fig. 8a: Electron micrograph of a part of a previtellogenic oocyte (PO) of unfed *A. persicus*. LY, lysosomes; S, rickettsia-like microorganisms; TP, tunica propria; V, vacuole (x 16525).

Fig. 8b : As in Fig. 8a but higher magnification showing mitochondria (M) (x 42750).

Fig. 9a : Electron micrograph of a part of a previtellogenic oocyte (PO) of unfed *A. persicus* showing the structure of the tunica propria (TP). MV, microvilli (x 38750).

Fig. 9b: As in Fig. 9a but directly after feeding showing the development of surface microvilli (MV) . cv, coated vesicles ; TP, tunica propria (x 22250).

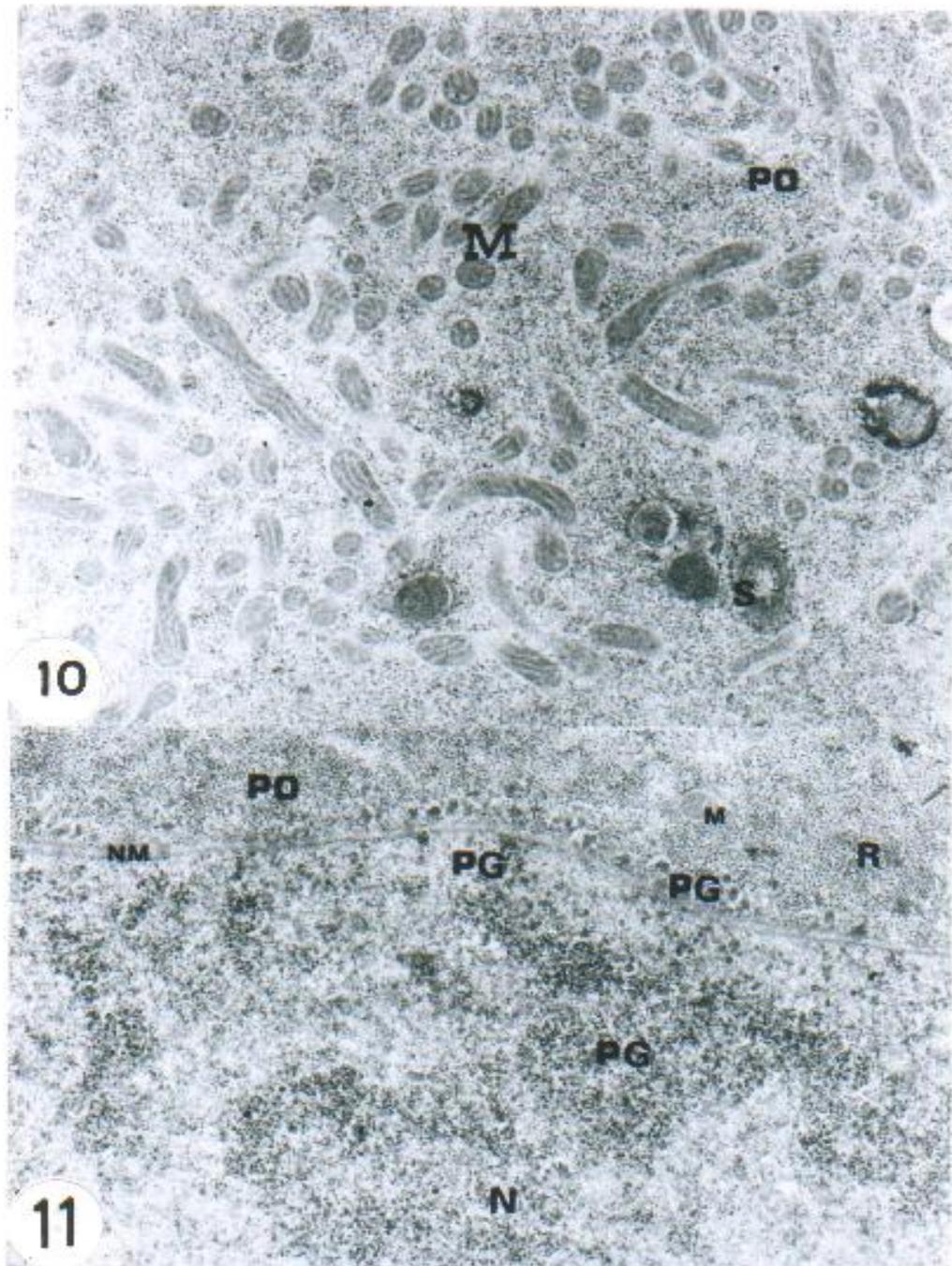


Fig. 10: Electron micrograph of a part of a previtellogenic oocyte (PO) of *A. persicus* directly after feeding showing multiplication of mitochondria (M). S, rickettsia-like microorganisms (x 22250).

Fig. 11: As in Fig.10 but showing the passage of perichromatin granules (PG) from the nucleus (N) toward the cytoplasm through the nuclear membrane (NM). M, mitochondria; R, ribosomes (x 22250).

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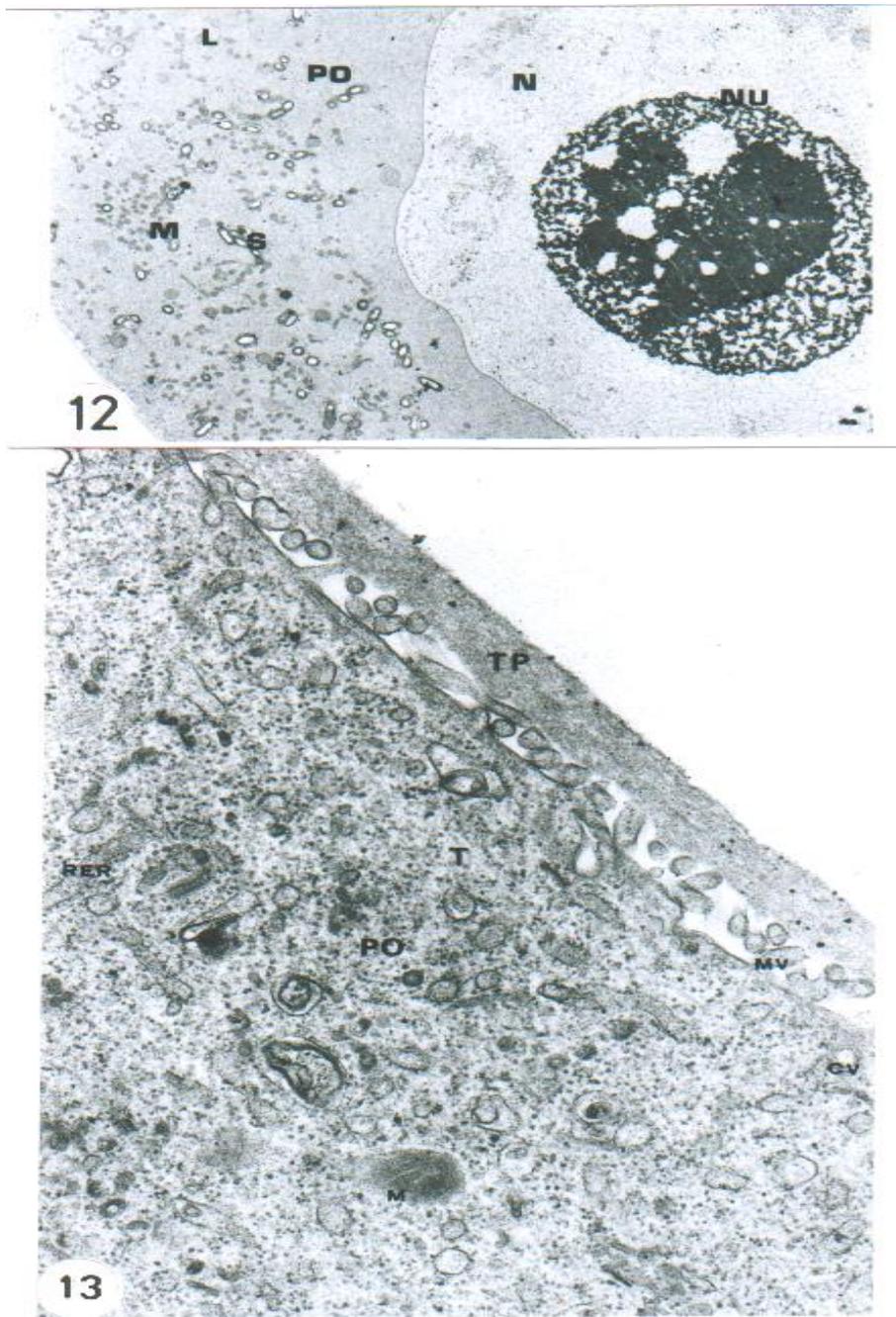


Fig. 12:As in Fig. 11 but 4 days after feeding showing the enlarged nucleus (N) containing fine euchromatin granules in addition to large nucleolus (NU). L, lipid droplets; M, mitochondria; S, rickettsia-like microorganisms (x 3300).

Fig.13: Electron micrograph of a part of a previtellogenic oocyte (PO) of *A. persicus* 4 days after feeding showing the formation of numerous surface microvilli (MV) underlined with coated vesicles (cv) and microtubules (T). M, mitochondria; RER, rough endoplasmic reticulum; TP, tunica propria (x 38750).

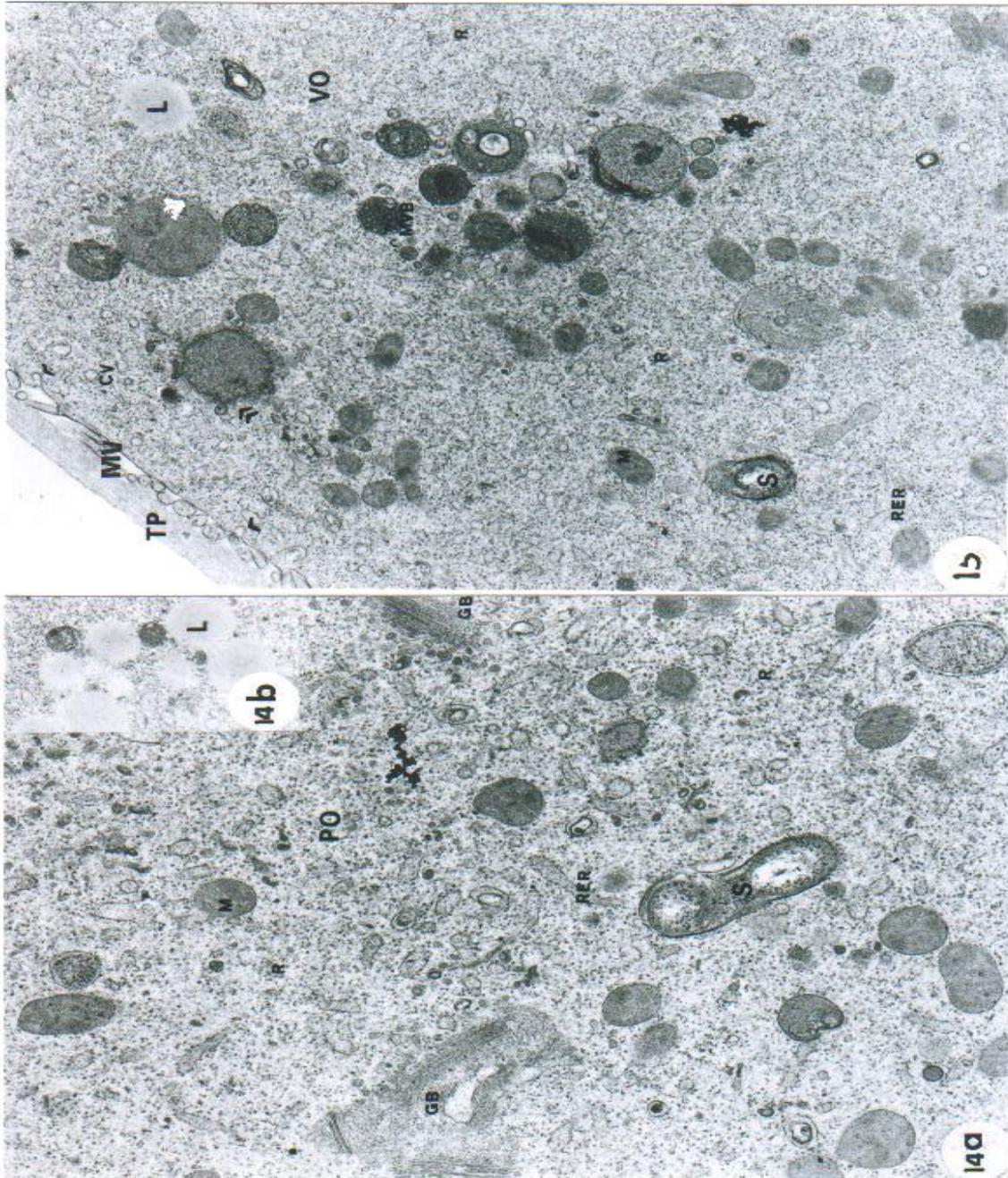


Fig. 14a: Electron micrograph of a part of a previtellogenic oocyte (PO) of *A. persicus* 4 days after feeding showing development of several groups of Golgi bodies (GB). M, mitochondria; R, ribosomes; RER, rough endoplasmic reticulum; S, rickettsia-like microorganisms (x 28750).

: **Fig.14a** but showing lipid droplets (L) (x 22250).

Fig. 15: Electron micrograph of a part of a vitellogenic oocyte (VO) of *A. persicus* 4 days after feeding showing formation of multivesiculate bodies (MVB). cv, coated vesicles; L, lipid droplets; M, mitochondria; MV, microvilli; R, ribosomes; RER, rough endoplasmic reticulum; S, rickettsia like microorganisms; TP, tunica propria; single arrowheads, micropinocytotic pits; double arrowheads, fusion of coated vesicles to multivesiculate body (x 22250).

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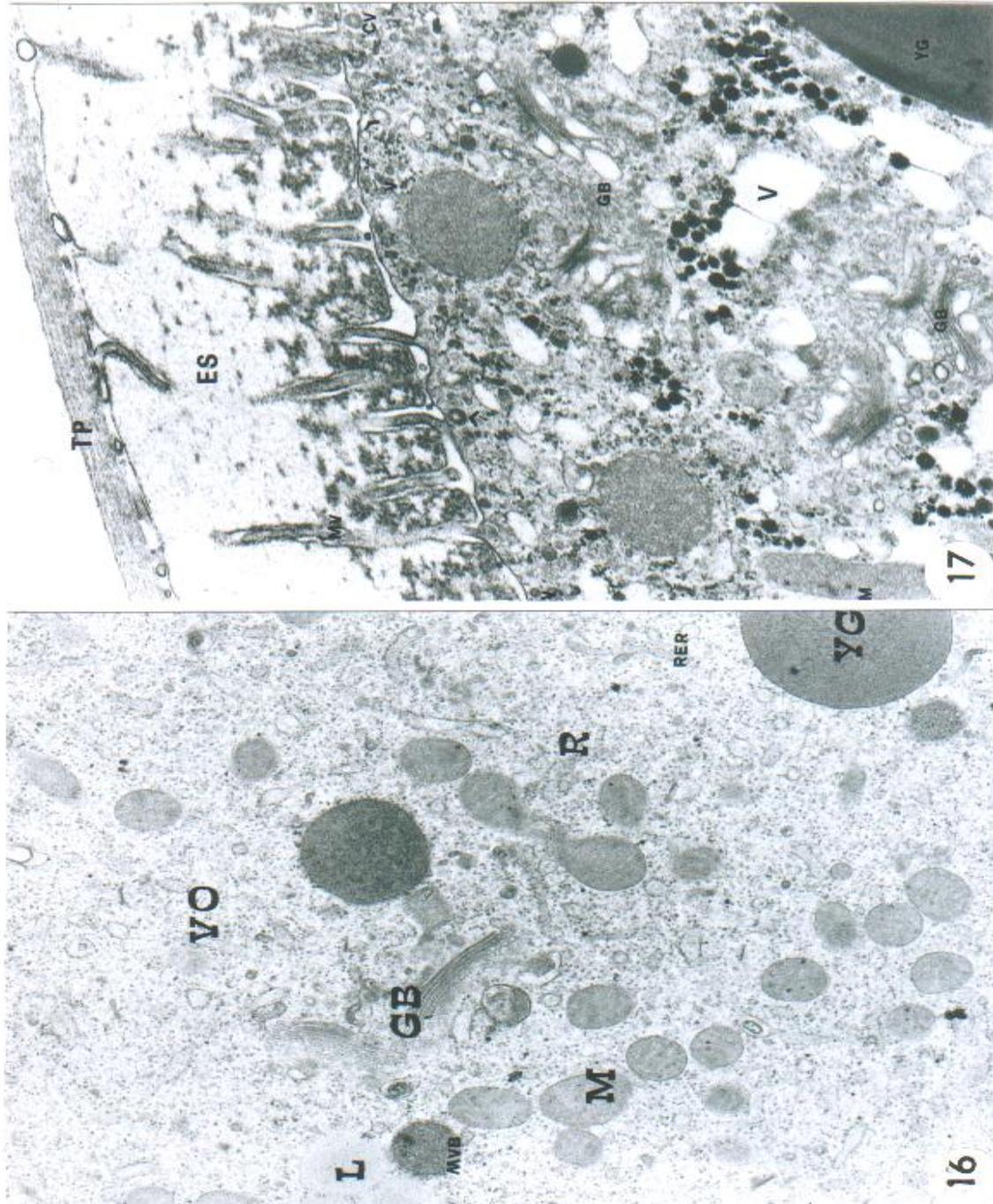


Fig. 16: Electron micrograph of a part of a vitellogenic oocyte (VO) of *A. persicus* 4 days after feeding showing fully formed yolk granules (YG). GB, Golgi bodies; L, lipid droplets; M, mitochondria; MVB, multivesiculate body; R, ribosomes; RER, rough endoplasmic reticulum (x 28750).

Fig. 17: Electron micrograph of a part of a vitellogenic oocyte (VO) of *A. persicus* 6 days after feeding showing formation of egg shell (ES) and inclusion of numerous Golgi bodies (GB). cv, coated vesicles; M, mitochondria; MV, microvilli; TP, tunica propria; V, vacuole; v, small vesicles; arrowheads, micropinocytotic pits (x 28750).

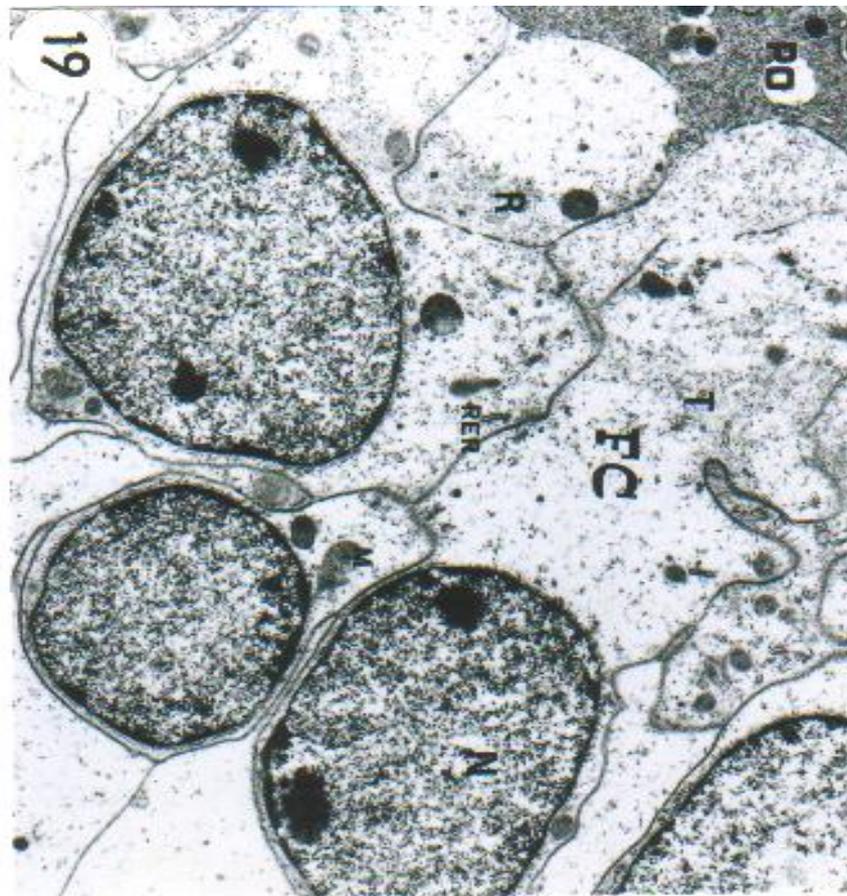
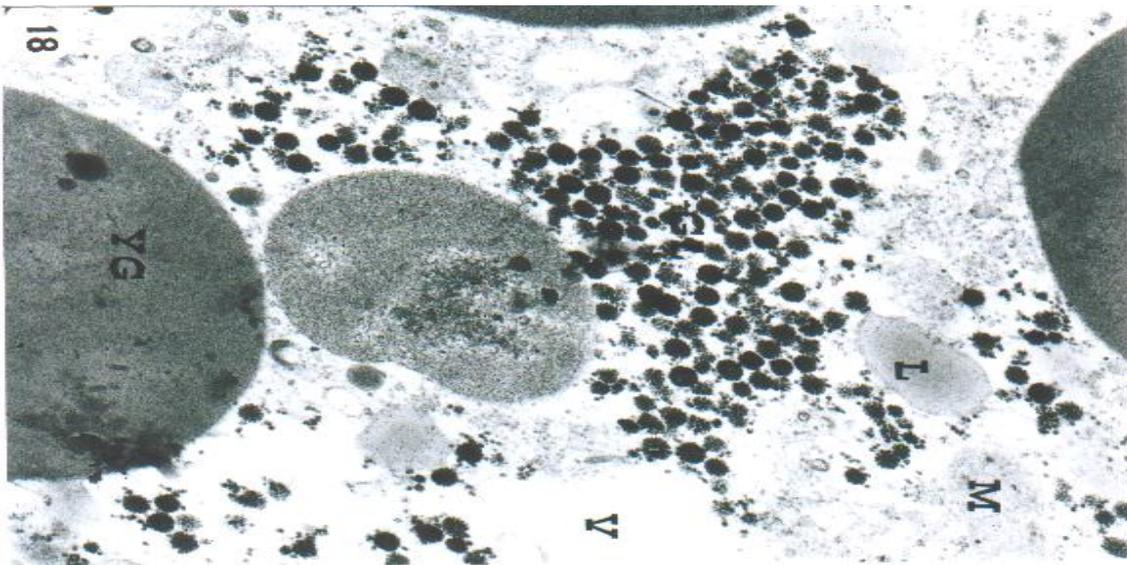


Fig. 18: Electron micrograph of a part of a vitellogenic oocyte (VO) of *A. persicus* 6 days after feeding showing narrow cytoplasmic areas occupied with large glycogen particles (GL), vacuoles (V) and lipid droplets (L) between yolk spheres (YG) (x 38750).

Fig. 19: Electron micrograph of funicle cells (FC) and their attachment to the previtellogenic oocyte (PO) of unfed *A. persicus*. M, mitochondria; N, nucleus; R, ribosomes; RER, rough endoplasmic reticulum; T, microtubules (x 9750).

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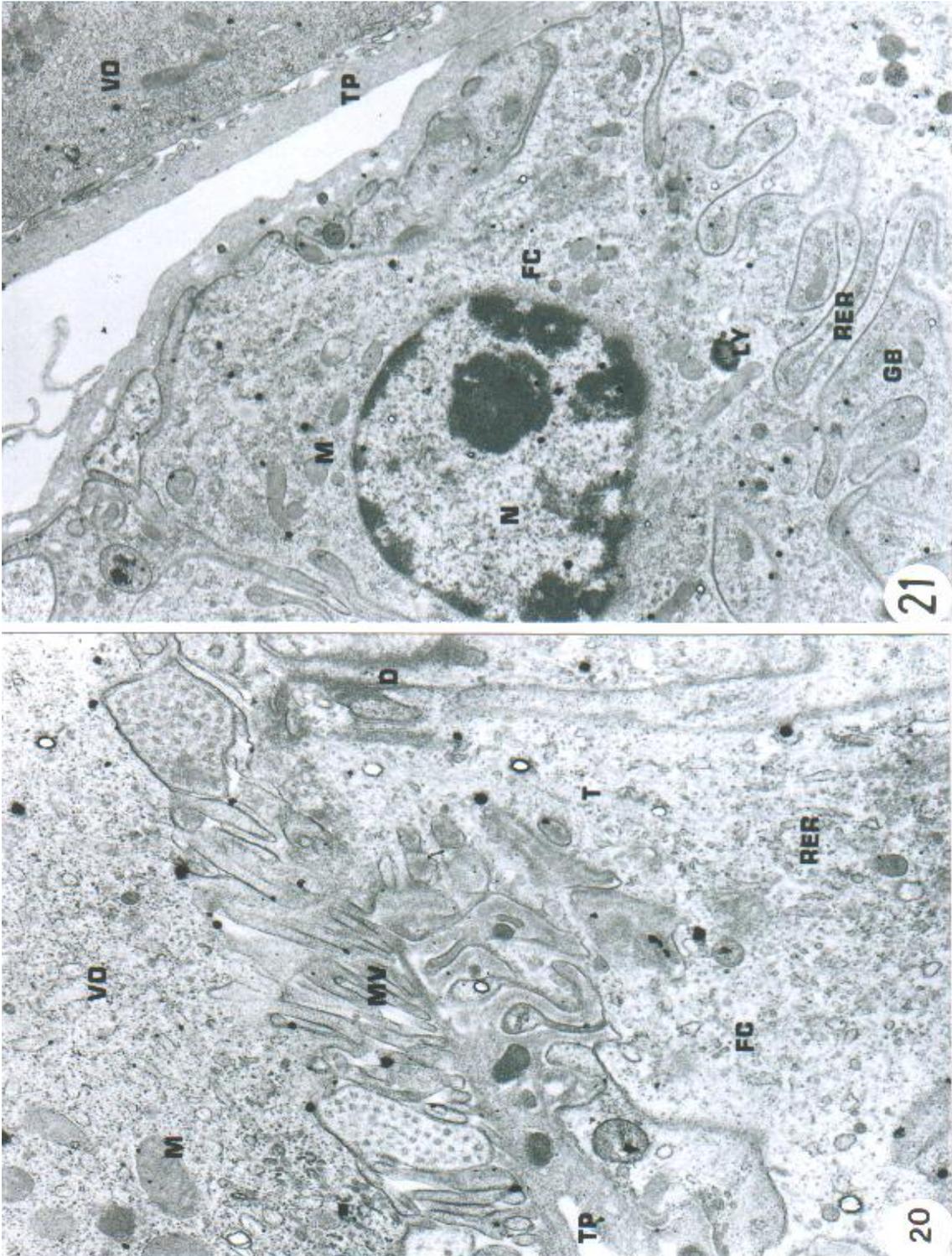


Fig. 20: Electron micrograph of the funicle cell (FC)-vitellogenic oocyte (VO) attachment site of *A. persicus* 4 days after feeding showing desmosomes (D) between funicle cells adjacent to the oocyte in addition to numerous microvilli (MV). M, mitochondria; RER, rough endoplasmic reticulum; T, microtubules; TP, tunica propria (x 28750).

Fig. 21: Higher magnification of a funicle cell (FC) connected to vitellogenic oocyte (VO) of *A. persicus* 4 days after feeding. GB, Golgi bodies; LY, lysosomes; M, mitochondria; N, nucleus; RER, rough endoplasmic reticulum; TP, tunica propria (x 16525).

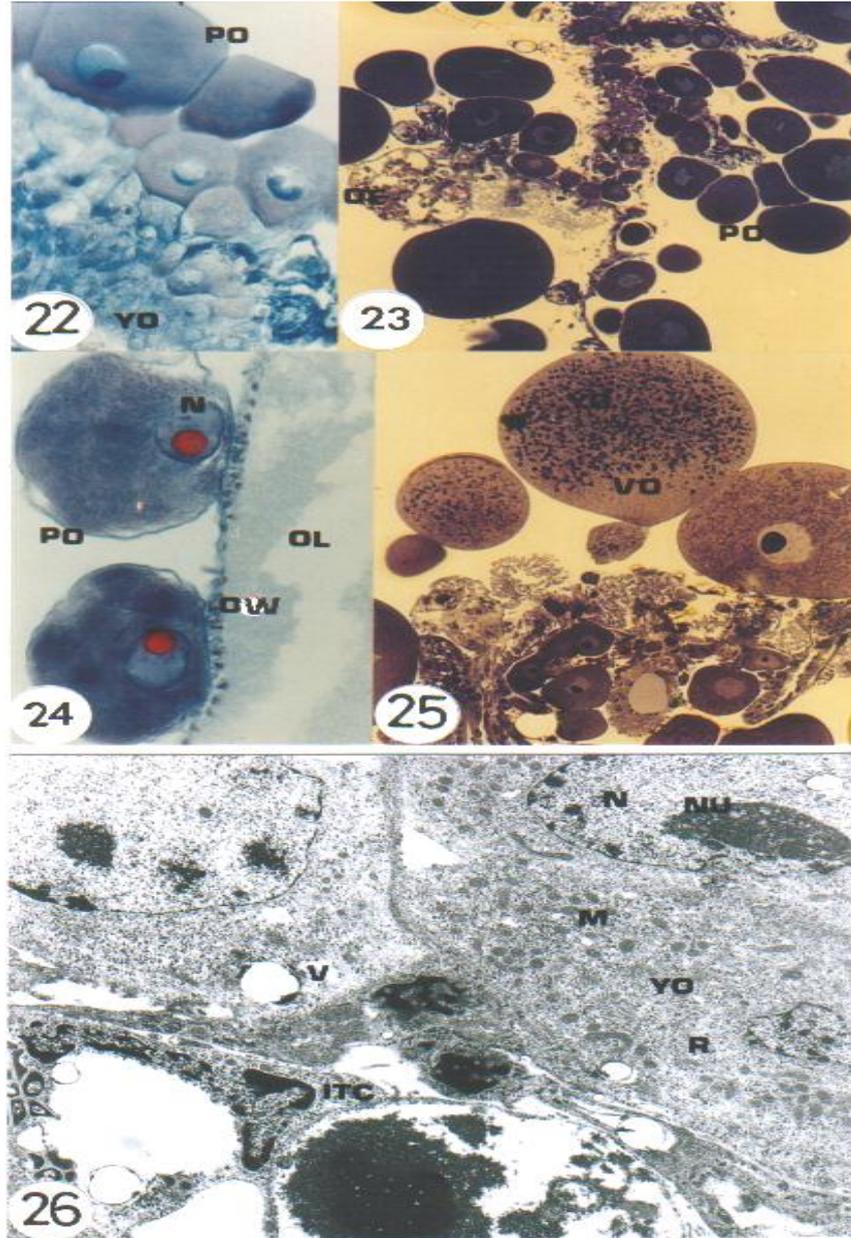


Fig. 22: Transverse section passing through the ovary of *A. persicus* directly after feeding on an ivermectin treated host showing the accumulation of young (YO) and previtellogenic (PO) oocytes (MT stain, x 312.5).

Fig.23: Semithin section of the ovary of *A. persicus* directly after feeding on an ivermectin treated host showing the damage inthe ovarian epithelium (OE) (MB stain, x 200).

Fig. 24: As in Fig. 22 but 6 days after feeding showing the ovarian lumen (OL) filled with a fluid and the ovarian wall (OW) stretched carrying previtellogenic oocytes (PO) (MT stain, x 312.5).

Fig. 25: As in Fig. 23 but 6 days after feeding showing the appearance of small yolk granules (YG) in few vitellogenic oocytes (VO) (MB stain, x 200).
Fig. 61: Transverse section passing through the ovary of *A. persicus* directly after feeding on an ivermectin treated host. PO, previtellogenic oocyte (PAS stain counterstained with Hx stain, x 200).

Fig. 26: Electron micrograph of young oocytes (YO) and interstitial cells (ITC) of *A. persicus* directly after feeding on an ivermectin treated host. M, mitochondria; N, nucleus ; NU, nucleolus ; R, ribosomes; V, vacuole (x 9750).

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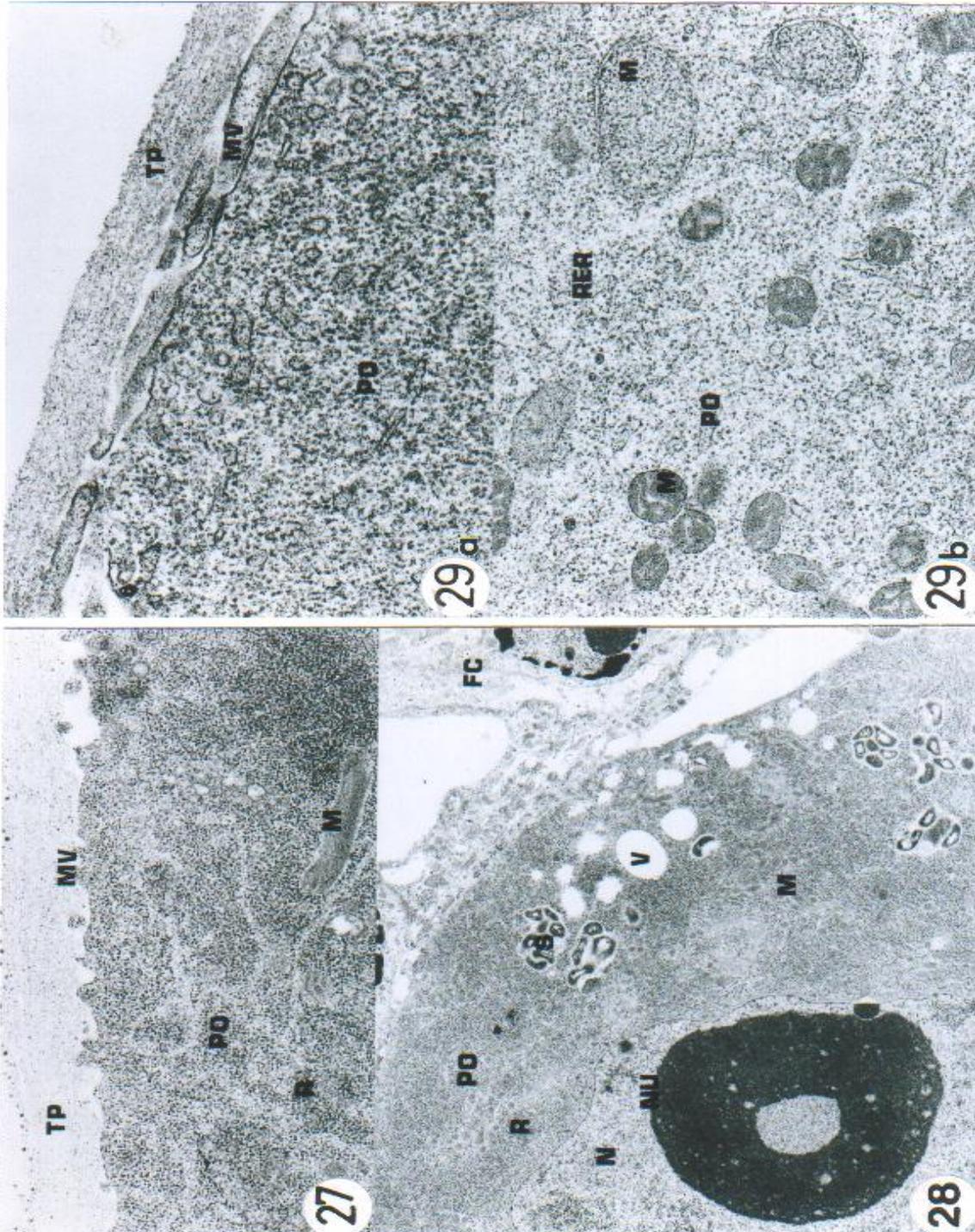


Fig.27: Electron micrograph of a part of a previtellogenic oocyte (PO) of *A. persicus* directly after feeding on an ivermectin treated host. M, mitochondria; MV, microvilli; R, ribosomes; TP, tunica propria (x 28750).

Fig.28: As in Fig. 27 but showing vacuolation (V) near the oocyte (PO)-funicle cells (FC) attachment site. M, mitochondria; N, nucleus; NU, nucleolus; R, ribosomes; S, rickettsia-like microorganisms (x 9750).

Fig. 29a: Electron micrograph of a part of a previtellogenic oocyte (PO) of *A. persicus* 6 days after feeding on an ivermectin treated host showing fewer microvilli (MV). TP, tunica propria (x 52500).

Fig. 29b: As in Fig. 29a but showing mitochondrial (M) degeneration. RER, rough endoplasmic reticulum (x 38750).

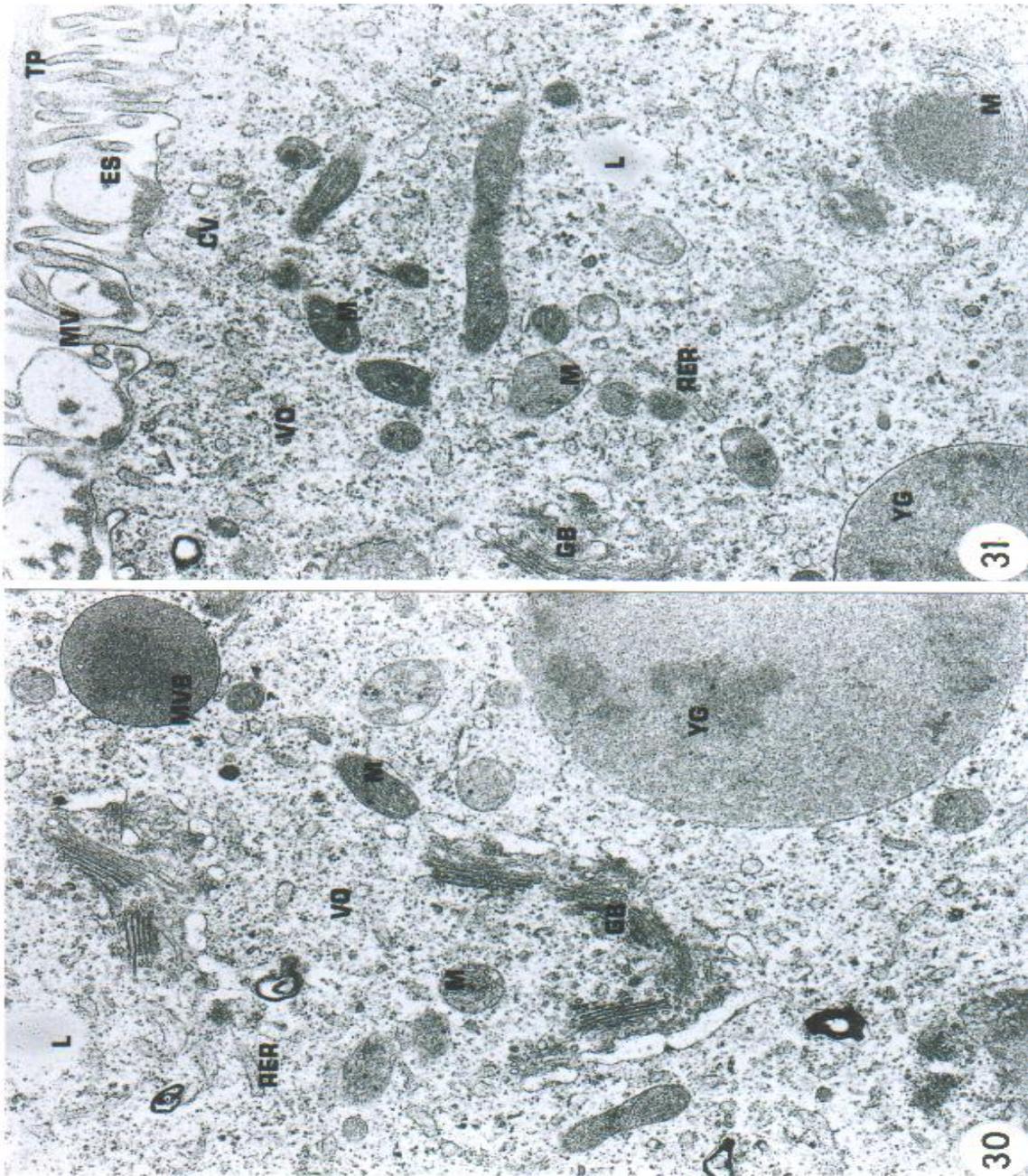


Fig. 30: Electron micrograph of a part of a vitellogenic oocyte (VO) of *A. persicus* 6 days after feeding on an ivermectin treated host showing formation of multivesiculate bodies (MVB), yolk granules (YG) and several Golgi bodies (GB). L, lipid droplets; M, mitochondria; RER, rough endoplasmic reticulum (x 28750).

Fig. 31: Electron micrograph of a part of a vitellogenic oocyte (VO) of *A. persicus* 6 days after feeding on an ivermectin treated host showing formation of egg shell (ES) and yolk granules (YG) as well as sharing of degenerated mitochondria (M) and rough endoplasmic reticulum (RER) in yolk formation. cv, coated vesicles; GB, Golgi bodies; L, lipid droplets; MV, microvilli; TP, tunica propria (x 28750).

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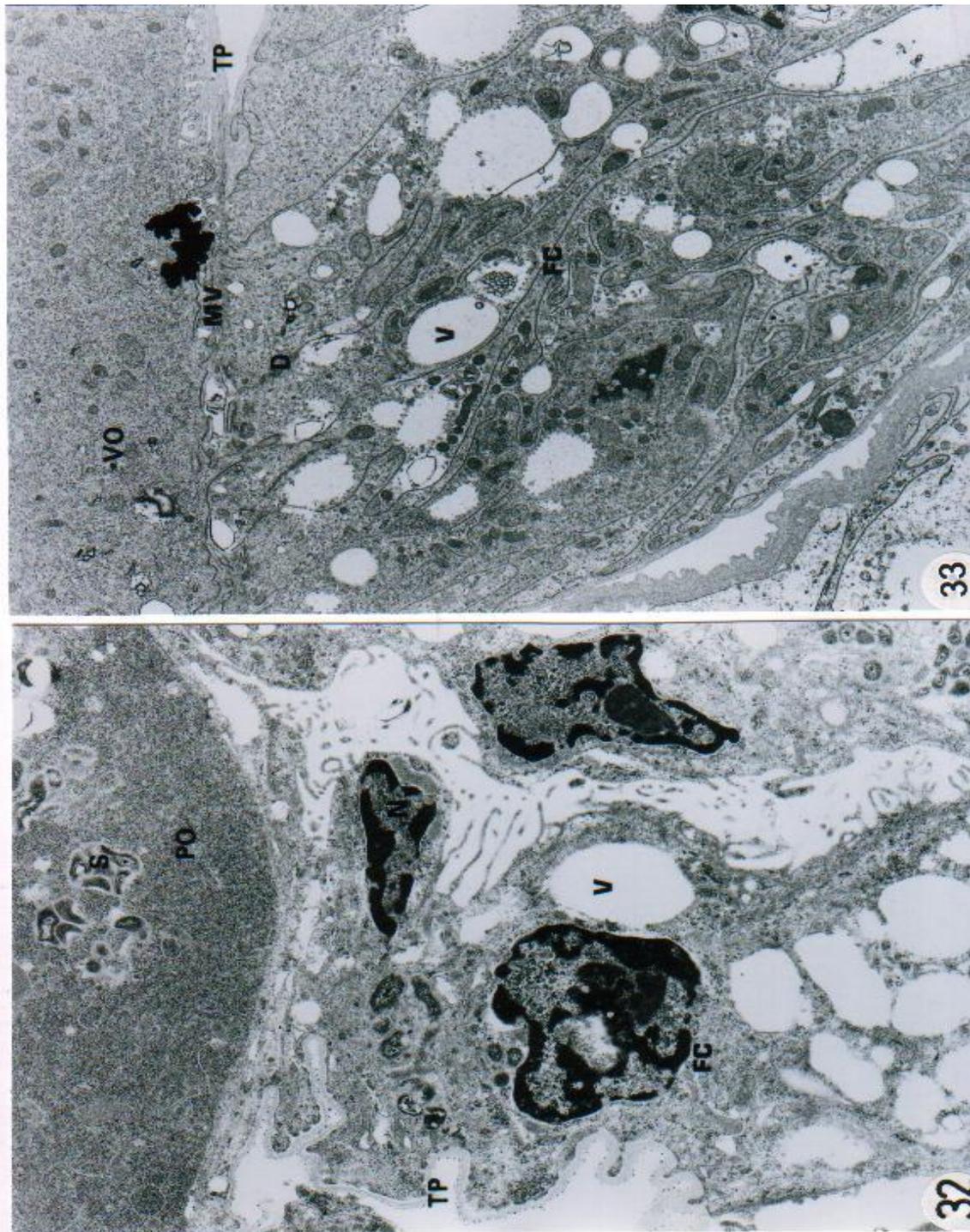


Fig.32: Electron micrograph of the funicle cells (FC) attached to previtellogenic oocyte (PO) of *A. persicus* directly after feeding on an ivermectin treated host. N, nucleus; S, rickettsia-like microorganisms; TP, tunica propria; V, vacuole (x 13000).

Fig. 33: Electron micrograph of the funicle cells (FC) attached to vitellogenic oocyte (VO) of *A. persicus* 6 days after feeding on an ivermectin treated host. D, desmosomes; MV, microvilli; TP, tunica propria; V, vacuole (x 9750).

Discussion

General structure:

In the present study, the position and structure of the single, transverse ovary in *A. persicus* lying in the posterior half of the body, with ova at different developmental stages protruding onto its surface is identical to those described in other argasids (Balashov, 1972; El Shoura, 1975; El Shoura, 1988).

The histological and ultrastructural examination of *A. persicus*, revealed that the ovarian wall consists of an inner epithelial layer enclosing a narrow central lumen and an outer delicate connective tissue sheath or tunica propria similar to that described in other ticks (Roshdy, 1967). The ovary before feeding contains ova in early stages of development. Tube-like passageway termed pedicels, connect the epithelium and the ovarian lumen with oocytes protruding onto the ovary surface. However, following a blood meal, the oocytes in *A. persicus* as in other argasids and ixodids grow at various rates and the previtellogenic and vitellogenic stages of primary oocytes together with oogonia are thus observed in the same ovary. At any rate, a physiological trigger apparently is necessary to stimulate vitellogenesis (Khalil, 1969). Feeding and engorgement apparently provide a stimulus mediated via the central nerve mass that initiates mitotic activity (Brinton and Oliver, 1971). The fact that oocytes of different developmental stages can be encountered at any time ensures the prolongation of vitellogenesis and hence oviposition period. This prolongation may have definite advantages for efficient production of egg batches allowing enough time for handling of eggs during water proofing by Gene's organ and permitting extracellular

source of yolk material to synthesize and export the precursors of yolk.

The general course of oocyte development and growth is also similar in the present studied tick to that described earlier in argasids (Roshdy, 1967; Khalil, 1969; Brinton and Oliver, 1971; El Shoura *et al.*, 1989). Balashov (1972) described *A. persicus* oogenesis in detail in five stages. Stage one is a period of "small growth" of primary oocytes and is always present in the ovary. Stage two is the period of great nuclear and cytoplasmic growth and ends with the appearance of the first yolk granules in the cytoplasm. Stages three and four are characterized by the appearance of yolk granules and end with ovulation. Stage five begins with mature eggs "coming into the lumen of the ovary".

In the present study, young oocytes greatly resemble stage I of Balashov's classification (1972) which resulted from oogonial division and during which the oocyte nucleus and cytoplasm increase only slightly (Diehl *et al.*, 1982). The fine structure of primary oocytes and interstitial cells of unfed *A. persicus* in the present study is basically similar to that in other ticks viz in unfed (Aeschlimann and Hecker, 1970; Raikhel, 1978; Balashov, 1983 El Shoura *et al.*, 1989). Primary oocytes of unfed *A. persicus* are found to demonstrate few small mitochondria and rough endoplasmic reticulum. Following feeding, mitochondria become numerous and fill most of the oocyte cytoplasm. The nucleus becomes large and is filled with fine granules of euchromatin, masses of heterochromatin and large dense nucleolus.

Previtellogenic oocytes observed in the present study correspond to stage II of Balashov's classification in which the cytoplasm greatly increases and

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lasts till the appearance of the first yolk granules. The ultrastructure of previtellogenic oocytes, which corresponds to stage II of Balashov's classification (1972), reveals a gradual increase in mitochondria and surface microvilli. Brinton and Oliver (1971) revealed that morphogenic changes of the mitochondria exhibit their involvement in the production of membrane-limited multivesiculate bodies. Rough endoplasmic reticulum and Golgi bodies are similarly developed and increased when compared to the unfed condition and microvilli become longer and more numerous. Surface microvilli in the growing oocytes are thought to facilitate micropinocytosis of nutrient material from the haemolymph (Diehl *et al.*, 1982). Towards the end of the great cytoplasmic growth phase, numerous lipid droplets are observed. Lipids observed between yolk granules in the vitellogenic phases of ticks were previously documented in *A. arboreus* (El-Shoura *et al.*, 1989). The nucleus of the previtellogenic oocytes exhibits a notable increase within the few days following feeding. Such increase was also reported in *A. arboreus* (El-Shoura *et al.*, 1989). Also, aggregates of perichromatin granules are observed beneath the nuclear membrane and pass through its pores to the cytoplasm. These granules were histochemically demonstrated as ribonucleoproteins and considered to be ribosomal precursors (Diehl *et al.*, 1982).

Vitellogenic oocytes characterized by the formation of yolk droplets are similar to stages III and IV of Balashov's classification. The vitellogenic phases of oocyte growth begin with the appearance of the first yolk granules and ends with ovulation (Diehl *et al.*, 1982). They are similar to stages III and IV of Balashov classification. The yolk formation in tick oocytes may

originate from both intracellular and extracellular sources (Diehl *et al.*, 1982; Balashov, 1983; El-Shoura *et al.*, 1989). The intracellular source mainly depends on the activities of the internal organelles as rough endoplasmic reticulum, Golgi bodies and mitochondria. In the present study, they are well developed and become more numerous within the few days following feeding. The fusion of small vesicles derived from Golgi bodies and rough endoplasmic reticulum in addition to mitochondria, ribosomes and perichromatin granules, emitted from the nucleus, into multivesicular bodies (yolk granule precursor) supports the intraoocytic source of yolk formation. The multivesicular bodies are mainly formed of proteins (Brinton and Oliver, 1971; Balashov, 1983). Similar involvement of mitochondria, dictyosomal vesicles, nuclear emissions and ribosomes in the formation of primary yolk bodies have been observed in several insects and arachnids (Brinton and Oliver, 1971). The extraoocytic source of yolk is evident by the formation of surface microvilli underlined with several micropinocytotic pits and vesicles. These vesicles subsequently appear to fuse with each other and with multivesicular bodies and transformed into large homogeneous yolk spheres through repeated fusions and dissolution of vesicle membranes. Similar observations were obtained in *R. bursa* (Hecker and Aeschlimann, 1970), *D. andersoni* (Brinton and Oliver, 1971), *Amb. hebraeum* (Diehl *et al.*, 1982) and *A. arboreus* (El-Shoura *et al.*, 1989). In the present study, large rosette-like glycogen deposits and lipid droplets accumulate in the oocyte cytoplasm between yolk granules. Similar results were obtained in previous ultrastructural investigations (Hecker and Aeschlimann, 1970; El Shoura *et al.*, 1989).

Ultrastructural examination in the present study reveals that the funicle cells carrying oocytes are irregular in shape, fastened to each other by several desmosomes and collectively coated with tunica propria. They contain large nuclei, few cisternae of rough endoplasmic reticulum, small mitochondria and microtubules. Microtubules increase during the process of vitellogenesis particularly near the oocyte-funicle cells attachment site. This is thought to enhance the mechanical role of such cells as suggested by Diehl *et al.* (1982). Desmosomal junctions connecting these cells also supported this function.

Deposition of egg-shell in the present study is found to begin slightly after the start of yolk formation. This greatly coincides with results obtained by Diehl *et al.* (1982) on *Amb. hebraeum* who recorded its beginning during Balashov's stage III of oocyte development and completion during stage IV (stages of yolk deposition). Egg-shell is mainly formed from vesicles, derived from Golgi bodies and endoplasmic reticulum, passes to the extracellular space below the tunica propria and is polymerized around microvilli. Participation of Golgi bodies in shell formation was previously suggested in *H. asiaticum* (Raikhel, 1978). The pattern of shell formation in the present study coincides with that described in *Amb. hebraeum* (Diehl *et al.*, 1982), *H. asiaticum* (Balashov, 1983) and *A. arboreus* (El Shoura *et al.*, 1989).

Effect of ivermectin:

In the present study on *A. persicus*, the single subcutaneous injection of the host by ivermectin at the dose rate 400 µg/kg affected growth and development of the oocytes following feeding, where the size of ova and their nuclei were comparatively smaller

in the treated ones. Vitellogenesis was not inhibited totally, however the dose affected yolk formation causing disrupted vitellogenesis, accompanied by a noticeable depletion and uniformity of the formed yolk particles and the absence of glycogen during the examined period.

Ivermectin was also found to induce the presence of a fluid in the ovarian lumen which caused its dilatation and hence the ovarian wall appears considerably stretched when compared with the untreated group.

Ultrastructural observation revealed a notable decrease in microvilli, micropinocytotic vesicles and perichromatin granules that traverse the nuclear membrane. Vacuolation was observed in parts of the cytoplasm as well as degeneration of mitochondria and rough endoplasmic reticulum.

However, ivermectin did not completely prevent the formation of egg shell where its thickness measured about half of the untreated one. It also caused the decrease in the number of coated vesicles that share in egg-shell formation.

Data concerning the histopathological effect of ivermectin on ticks are rare, since most workers concentrated on its systematic efficacy and on ticks biological response to various levels of the drug (Drummond *et al.*, 1981; Lancaster *et al.*, 1982; Campbell *et al.*, 1983; Drummond, 1985). Drummond *et al.* (1981) mentioned that ivermectin do not block vitellogenesis specifically. First they report that ivermectin does not affect the appearance of gonadal tissues in small ticks. Secondly, when a few small females were transferred from an ivermectin treated to a control calf, they reattached, engorged and laid small-to moderate-sized egg masses that had a reduced hatch. Kaufman *et al.* (1986) studied the effect of ivermectin

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on feeding, salivary gland fluid secretion and fecundity in some ixodid ticks. When injected into engorged ticks, the drug increased oviposition latency and reduced fecundity. On the other hand, ivermectin was reported to reduce the rate of vitellogenesis and follicle development, in fire ants (Lofgren and Williams, 1982), producing marked histopathological effects in the ovaries (Glancey *et al.*, 1982). Similar effects are also described in the mosquito *Aedes aegypti* (Mahmood *et al.*, 1991) after the ingestion of blood containing the drug. The authors described histological changes occurring as degeneration of primary follicles, formation of ovarian dilatations, significant reduction of the rate of vitellogenesis and follicle development, expressed by little or no yolk deposition. Similar histopathological effects were reported in the nematodes *Molinema dessetae* (Duarte *et al.*, 1994) and *Capillaria hepatica* (El-Nassery *et al.*, 1996).

Ultrastructural changes including degeneration, vacuolization of the cytoplasm and condensation of nuclei were reported in muscle cells as well as smoothening, ulcer formation and stretching in some parts of the cuticle due to ivermectin treatment in *O. volvulus* (Wildenburg *et al.*, 1994), *C. hepatica* (El-Gebaly *et al.*, 1996) and *Trichinella spiralis* (El-Azzouni, 1997). Moreover Perez *et al.* (2001) stated that the cestoeidal effect of ivermectin on the tapeworm, *Echinococcus granulosus* is through its effect on the tegument and causing a state of depolarization followed by rostellar disorganization, rigid paralysis and, eventually loss of viability. El-Gebaly *et al.* (1996) and El-Nassery *et al.* (1996) demonstrated shrinkage and deformation in the egg-shell of *C. hepatica* together with some irregularities and

longitudinal grooves as well as fragmentation of some surface pillars.

Ivermectin exerted its therapeutic effect by action on ligand-gated membrane ion-channels particularly glutamate gated channels which have so far been found only in invertebrates (Martin *et al.*, 1998; Perez *et al.*, 2001). Ivermectin increased inward current through membranes of *Xenopus laevis* oocytes. Such currents were activated in presence of glutamate and desensitized in the continued presence of glutamate (Arena *et al.*, 1992). The latter authors proposed that the glutamate-sensitive chloride current and the avermectin-sensitive chloride current were mediated via the same channel. Ivermectin was also capable of binding to the membrane transporter-P-glycoprotein in mouse and human (Schinkel *et al.*, 1995; Brown, 1998) and to P-glycoprotein in nematodes (Smith and Prichard, 2002). The lipophilic nature of ivermectin was previously observed by its ability to unite with the lipid phase of muscle membranes of *Ascaris suum* (Martin and Kusel, 1992).

Nothing is known about the sites or mechanism of action of the avermectins in ticks. In the nematode, *Ascaris*, avermectin causes paralysis by blocking transmission between interneurons and excitatory motoneurons in the ventral nerve cord; while the γ -aminobutyric acid (GABA)-antagonist, picrotoxin, reverses avermectin-induced blockade (Kass *et al.*, 1980). Other studies indicate that avermectins stimulate GABA systems in general: (1) they stimulate GABA-mediated chloride conductance in a number of preparations (Mellin *et al.*, 1983); (2) they bind avidly to dog brain synaptosomes (Pong and Wang, 1980); and (3) they stimulate release of GABA from rat brain synaptosomes (Pong *et*

al., 1980). Avermectin also has effects on benzodiazepine receptors (Supavilai and Karobath, 1981), and on presynaptic GABA receptors (Pong and Wang, 1979). The fact that avermectin induces immobilization in helminths as well, suggests that avermectins cause their anti-parasite effect by interacting with a putative GABA pathway in helminths.

In ticks, it seems probable that an insect-like juvenile hormone is used as a gonadotropic hormone to regulate vitellogen production and other aspects of oogenesis (Diehl *et al.*, 1982). Experiments have also implicated the synganglion as the seat of an endocrine mechanism regulating oogenesis (Shanbaky and Khalil, 1975). Gabbay and Warburg (1976) suggested that a neurosecretory product is involved in the first step of an endocrine mechanism regulating the digestion or mobilization of blood-meal proteins, the initiation of vitellogenin production and its release into the haemolymph and/or its incorporation into developing oocytes. Data from the present study suggests a partial blockage of the release of vitellogenetic hormone as indicated by disrupted synthesis and uptake of yolk protein in the developing oocytes. However, still more work is needed to assess the exact titre of vitellin in the ovary in an attempt to fully understand ivermectin exact mode of action.

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**التغيرات التركيبية الدقيقة والهستولوجية المستحدثة بعقار الأفرميكيتين فى
مبيض القراد أرجس برسيكس بعد التغذى
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يتكون جدار المبيض فى القراد أرجس برسيكس من بويضات أولية فى ثلاث مراحل نمو مختلفة هى المرحلة الصغيرة ومرحلة ما قبل ظهور المح ثم مرحلة ظهور المح بالإضافة إلى الخلايا البينية . وقد لوحظ أنه بعد التغذية و النزواج قد زادت أحجام هذه الخلايا و الخلايا البينية و خاصة الخلايا الحبيبية التى تحمل البويضات الأولية وقد أظهرت عضياتها تغييرات ملحوظة ترتبط بتكوين المح وقشرة البيض . إستهدفت الدراسة فحص المظاهر التركيبية الدقيقة أثناء تكوين المح وقشرة البيض وقد وجد أن المح ينشأ من حويصلات صغيرة مشتقة من أجسام جولجى بالإضافة إلى أجسام صغيرة من الشبكة الأندوبلازمية المحببة وعوارض الميتوكوندريا. تتحد هذه الحويصلات مع بعضها لتكون أجسام متعددة الحويصلات تتحول فيما بعد إلى كرات المح. وقد أظهرت الدراسة أن قشرة البويضة تتكون من حويصلات منفصلة من أجسام جولجى والتى تلتحم مع الغشاء البلازمى وتخرج محتوياتها خارج جدار البويضة ما بين الغشاء القاعدى وطبقة القميص الأصيل. وايضاً لوحظ وجود تجمعات كثيرة من الجليكوجين وقطرات من الدهون بين كرات المح. كذلك أسفرت هذه الدراسة على أن معاملة العائل بالجرعة المختارة وهى 400 ميكروجرام/كيلوجرام من عقار الأفرميكيتين تمنع نمو البويضات حيث أن المبيض يظهر مكتظاً بالبويضات غير مكتملة النمو والتى تحيط بتجويف ضيق للمبيض أو يظهر جدار المبيض حاملاً البويضات مثل كيس منتفخ مملؤ بسائل. كما تظهر الخملات الدقيقة والحويصلات المغلفة أقل عدداً من الغير معاملة. كما لوحظ أيضاً حدوث تكسير وإضمحلال للميتوكوندريا والشبكة الإندوبلازمية داخل البويضات و الخلايا الطلائية الحاملة لها. ولقد وجد فى هذا البحث ان العقار يتسبب من حين لآخر فى منع تكوين حبيبات المح و غلاف البويضة . بينما فى البويضات القليلة المكتملة النمو تظهر أجسام جولجى غير متأثرة و غلاف البويضة أقل سمكاً وكثافة كما يظهر سيتوبلازم الخلايا الحاملة للبويضات مملؤ بفجوات كثيرة وعضيات أقل وضوحاً وأنوية منتفخة بدون غشاء نووى مميز ويمنع هذه العقار ظهور حبيبات الجليكوجين تماماً كما يتسبب أيضاً فى تقليل البروتينات والدهون فى البويضات. ويستخلص من هذا النتائج أن العقار يؤثر جزئياً على إنطلاق هرمون تكوين المح ويسبب صعوبة إمتصاص البروتين الخاص بتكوين البويضات.