VITELLOGENESIS AND EGG-SHELL FORMATION IN OVIPOSITING FEMALES OF THE TROMBICULID MITE *HIRSUTIELLA ZACHVATKINI* (SCHLUGER) (ACARIFORMES: TROMBICULIDAE)

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TROMBICULIDAE
CYTOLOGY
ULTRASTRUCTURE
OOGENESIS

SUMMARY: The developing oocytes in ovipositing females of the trombiculid mite *Hirsutiella zachvatkini* (Schluger, 1948) have been investigated during the course of vitellogenesis, using transmission electron microscopy. The external surface of the egg-shell was also examined under the scanning electron microscope. Oocyte growth may be divided into small growth, or previtellogenesis, and great cytoplasmic growth, or vitellogenesis. Growing oocytes subsequently migrate to the periphery of the ovaries, rupture the basal membrane and often protrude into the surrounding tissues. Late previtellogenic oocytes are rich in mitochondria and are characterized by the formation of curved microvilli on their surfaces. A fine-granular substance of low electron density, referred to as primary vitelline envelope, begins to deposit above the tips of microvilli. The short initial step of yolk accumulation is carried out intraoocytically by small Golgi complexes, scattered throughout the cytoplasm, during late previtellogenesis. Later in vitellogenesis, yolk precursors are accumulated by the oocyte from an extraoocytic source by high endocytotic activity, which is reflected in the presence of large numbers of coated pits and vesicles in the cortical ooplasm. Proteinaceous yolk bodies are formed by the fusion of endosomes and finally, as mature yolk bodies, they have crystalline cores. In addition to yolk bodies, lipid inclusions are deposited in the ooplasm. At the onset of vitellogenesis, Golgi complexes migrate peripherally to a position near a rim of cortical cytoplasm and give rise to many electron-dense vesicles which seem to deposit the secondary vitelline envelope, initially in the form of electron-dense spots between the microvilli. The external layer of the secondary vitelline envelope is formed by the secretory activity of the somatic cells which encircle the oocyte towards the end of vitellogenesis.

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RÉSUMÉ : Le développement des oocytes au cours de la vitellogenèse chez les femelles ovipares du trombiculidé *Hirsutiella zachvatkini* (Schluger, 1945) a été observé en microscopie électronique à transmission. La surface des œufs a été étudiée en microscopie électronique à balayage. Le développement des oocytes comprend une prévitellogenèse, ou petite croissance cytoplasmique, et une vitellogenèse, ou grande croissance cytoplasmique. Au cours de la croissance, les

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oocytes émigrent à la périphérie des ovaires, rompent la membrane basale et pénètrent dans les tissus cellulaires voisins. En prévitellogenèse avancée, les oocytes sont riches en mitochondries et sont caractérisés par la formation à leur surface de microvillosités. Au sommet des microvillosités commence à se déposer une substance finement granuleuse à basse densité électronique que l'on considère comme la membrane vitelline primaire. Au début, le dépôt de vitellus est assuré par un processus intraovocytaire placé sous l'action de petits appareils de Golgi librement répartis dans le cytoplasme en prévitellogenèse avancée. Puis au cours de la vitellogenèse, à partir de sources extraovocytaires, sous l'action endocytoplasmique intense de l'ooplasme cortical et en présence de très nombreuses cavités et vésicules de bordure, l'ovocyte amasse les substances qui donneront le vitellus. Des corpuscules vitellins protéiniques se forment en fusionnant avec des endosomes; ils montrent à la fin de leur maturation un noyau cristallin. En même temps que se forment les corpuscules vitellins, des inclusions lipidiques se déposent dans l'ooplasme. Dès le début de la vitellogenèse, les appareils de Golgi émigrent à la périphérie de l'oocyte pour se placer dans l'ooplasme cortical et former de nombreuses vésicules électroniquement denses. Ces vésicules fournissent au début, une membrane vitelline secondaire, par un processus d'exocytose, sous forme de taches sombres à la base des intervalles entre les microvillosités. La couche externe de cette membrane vitelline secondaire se forme lorsque s'achève la vitellogenèse, par une intense activité sécrétrice des cellules qui entourent l'oocyte.

Introduction

In spite of the fact that a number of electron microscopic studies have recently been made of the developing oocytes and the process of vitellogenesis in various species of mites (Reger, 1970; Witte, 1975; Balashov, 1979; Mothes & Seitz, 1981; Coons *et al.*, 1982; Feiertag-Koppen & Pijnacker, 1985; Witalinski, 1986, 1988; El Shoura *et al.*, 1989), no comprehensive studies have so far been carried out on the ultrastructural peculiarities of oogenesis for representatives of the group Trombiculidae. A great diversity exists in the time of formation and derivation of both the yolk bodies and the vitelline envelope(s) (extraoocytic, or heterosynthetic, and intraoocytic, or autosynthetic) of the oocytes, as well as in the classification of egg envelopes in the arthropods studied to date. This situation have stimulated our interest in the vitellogenesis and egg-shell formation of one of the highest and most specialized groups of trombidiform mites—the suborder Actinedida.

Knowledge of oocyte maturation in trombiculid mites, which are strongly characterized by larval parasitism on different vertebrate hosts, is essential to our understanding of their capabilities of being a generation-to-generation reservoir of pathogenic rickettsiae in nature, and of transovarial transmission of human and animal disease agents to their progenies.

The main aim of the present investigation is to describe the vitellogenic process, or great cytoplasmic growth, which includes the accumulation of yolk and the formation of the secondary vitelline envelope, in ovipositing females of the trombiculid mite *Hirsutiella zachvatkini* (Schluger, 1948). The ultrastructural peculiarities of previtellogenesis, or small oocyte growth, of this species have been reported in detail elsewhere (Shatrov, 1996).

Material and methods

Ovipositing females of *H. zachvatkini* used in this study were obtained from a laboratory culture maintained at the Laboratory of Parasitology, Zoological Institute, Russian Academy of Sciences, according to procedures described previously (Shatrov, 1993). As revealed by laboratory observations, females start to lay eggs only after being together with males for some time after their emergence from the tritonymphal

cuticle. Males are known to deposit spermatophores immediately after emergence, which are taken up by the females. This seems to serve as initial factor for oocyte growth and for the subsequent ovipositing cycle, which obviously can take place without males.

For transmission electron microscopy (TEM), repeatedly-fed ovipositing females of *H. zachvatkini* of the first laboratory generation, aged three to six weeks, were fixed intact in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.2-7.4) for 2-4 h. For better penetration of fixative solution, mites were carefully pierced with tiny, sharp, entomological pins in a caudal part of the body. They were then washed in several changes of 6.85% buffered sucrose, post-fixed in 1% osmium tetroxide in phosphate buffer containing 8.56% sucrose for 1-6 h to overnight, dehydrated in an alcohol and acetone series, and finally embedded in an araldite mixture. Serial ultra-thin sections were made on a LKB-III ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Tesla BS-500 transmission electron microscope at 60-90 kV.

For scanning electron microscopy (SEM), freshly laid eggs of the second generation were dried, after alcohol fixation, at the critical point of carbonic acid in a Hitachi HCP-2, and then covered with platinum layer in an Eiko-5 and examined in a Hitachi S-570 electron microscope at 20 kV.

Results

The oocyte growth of *H. zachvatkini* may be divided into small growth, or previtellogenesis, and great cytoplasmic growth, or vitellogenesis. During the final stages of the small growth, and especially during the course of vitellogenesis, the oocytes migrate to the periphery of the ovaries. They rupture the basal membrane (*tunica propria*) and are often seen to protrude into the surrounding tissues and haemocoelic space (Figs. 1, 2). The oocytes remain attached to the ovaries by means of a narrow connective region with somatic cells, provided with long mutual microvilli of both the somatic cells and the oocyte. This type of oogenesis may be recognized as belonging to the solitary type, which is also characteristic of some arachnids so far studied (Osaki, 1971; Seitz, 1971; Balashov, 1979; Kessel & Beams, 1980; Witalinski & Zuwala, 1981; El Shoura et al., 1989), including trombidiform mites (Feiertag-Koppen & Pijnacker, 1985).

Late previtellogenic oocytes are very large, round-to-oval cells, containing a centrally located clear nucleus with a diameter of up to 21 μm (Fig. 2). Their chromosomes are decondensed, and the reticulate, oval nucleolus ranges from 12 to 14 μm in diameter. The nuclear envelope is pierced by numerous pores, though apparent transport of the electron-dense ribonucleoprotein granules occurs at a moderate intensity during this period (Fig. 2). Oocytes are rich in mitochondria, with loosely-packed cristae and a matrix of varying density, that are scattered throughout the cytoplasm (Fig. 1). They are never associated with, or fused to, any dense granules or ribosomal aggregates, as has been reported for tick oocytes (Balashov, 1979; El Shoura *et al.*, 1989). Rough endoplasmic reticulum and other membrane profiles are typically absent in the ooplasm, although free ribosomes and polysomes are quite numerous (Fig. 3).

During the late previtellogenic period, a number of small Golgi complexes become distinguishable throughout the oocyte cytoplasm in the form of a few flattened cisternae, associated with several small vesicles (Fig. 3). Large electron-dense granules, which are considered to represent initial yolk bodies, may sometimes be seen in the vicinity of Golgi complexes. This reflects the short initial stage of the yolk formation by the intraoocytic (autosynthetic, endogenous) processes (Shatrov, 1996). During late previtellogenesis, the oolemma gradually forms short, curved microvilli. Above the tips of these a fine-granular substance of low electron density is formed (Figs. 2–5), which appears to represent the so-called primary vitelline envelope, since it is formed by the oocyte only (Reger, 1977). The maximum thickness of this envelope is approximately 2.0-2.5 μm.

Taking into account that the real accumulation of the main yolk masses occurs only after the formation of the primary vitelline envelope has been completed, it is legitimate to suppose that this envelope may play a role as a selective semipermeable barrier for macromolecules of the yolk protein precursors which enter the oocytes from the perivitelline space. The extraoocytic (heterosynthetic, exogenous) source(s) of these precursors are probably represented by the midgut.

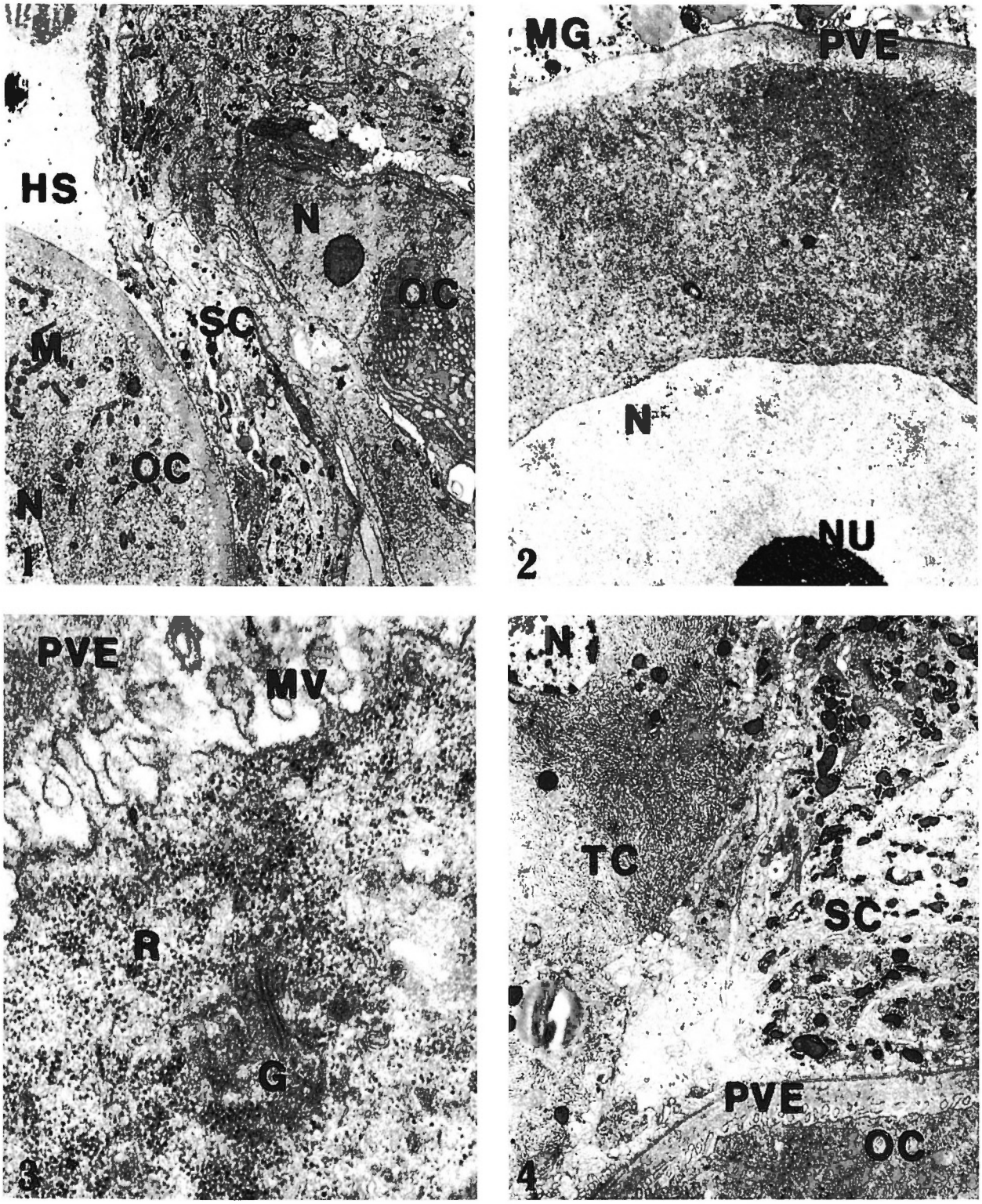


FIG 1: Dorso-medial portion of the ovary without delimiting basal membrane, occupied by previtellogenic oocytes at different stages (OC) and somatic cells (SC). Note haemocoelic space (HS), bordering the ovary tissue. N, nuclei, M, mitochondria. × 3 500.

FIG 2: Part of late previtellogenic oocyte, protruding into the midgut (MG), with primary vitelline envelope (PVE). Note large clear nucleus (N). Nu, nucleolus. × 6 000.

FIG 3: Peripheral part of late previtellogenic oocyte with Golgi complex (G) and numerous ribosomes (R). Note primary vitelline envelope (PVE) above the tips of microvilli (MV). × 45 000.

FIG 4: Portion inside the ovary with late previtellogenic oocyte (OC), provided with primary vitelline envelope (PVE) in contact with large masses of trophic (TC) and somatic (SC) cells. N, nucleus of trophic cell. × 7 500.

The latter borders the protruding oocytes and ovaries at their dorsal parts (Fig. 2) and contains large amounts of stored nutritive materials. Furthermore, these are trophic (nurse) cells of the ovaries, with strongly developed rough endoplasmic reticulum (WITTE, 1975; SHATROV, 1996) (Figs. 4, 8).

The beginning of vitellogenesis is marked by the appearance of the coated pits and vesicles and of the electron-dense vesicles in the narrow rim of the cortical ooplasm. These two pools of cortical vesicles appear to express two alternative routes of the cortical processes: exogenous accumulation of the yolk protein and endogenous formation of the secondary vitelline envelope.

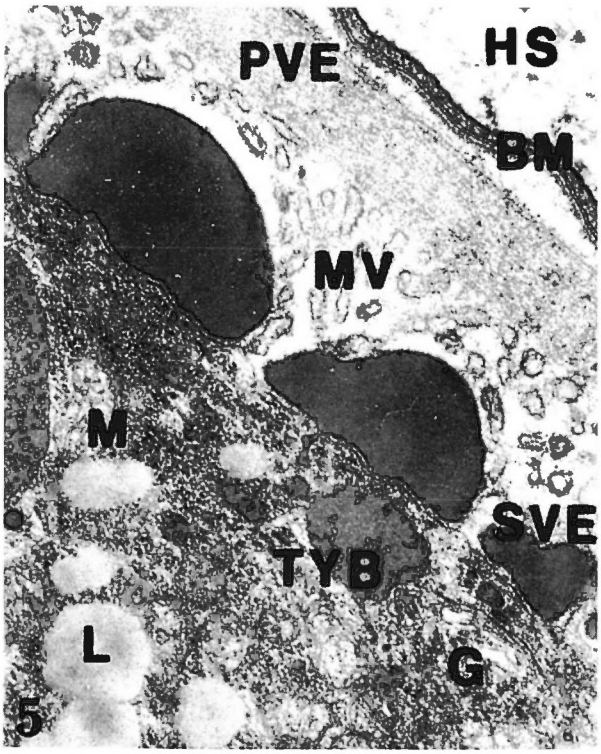
Electron-dense vesicles, whose diameter ranges from 0.18 to 0.21 μm, are found to originate from Golgi complexes that migrate peripherally at the onset of vitellogenesis (Fig. 5). Such impressive changes in the functioning of the Golgi complexes during the course of oogenesis have been also demonstrated for the growing oocytes of a horseshoe crab, *Limulus polyphemus* (DUMONT & ANDERSON, 1967), with the solitary type of oogenesis. In *H. zachvatkini* vitellogenic oocytes, the cortical electron-dense vesicles appear to be utilized in the formation of the secondary vitelline envelope. The latter comes to lie between the microvilli, initially in the form of homogeneous electron-dense patches which subsequently fuse (Figs. 5, 6). This process results in the formation of the uniform, electron-dense layer of the envelope, penetrated by relatively large electron-lucent pore canals containing a single microvillus (Figs. 6, 7). The developing secondary vitelline envelope displaces the primary vitelline envelope and apical plexus of microvilli more peripherally of the oocyte. Pore canals within the envelope provide the immediate access of yolk precursors to the oocyte from the extraoocytic space during the course of vitellogenesis.

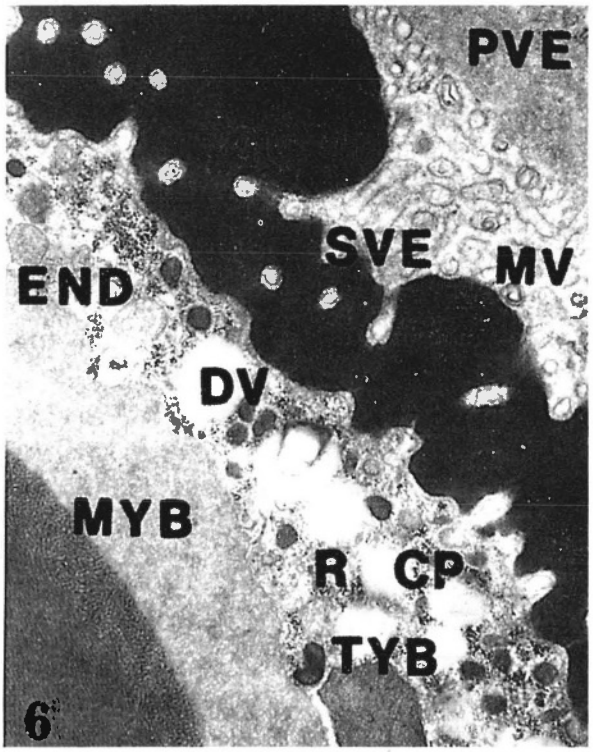
Simultaneously with the formation of the secondary vitelline envelope, the accumulation of yolk (vitellogenesis proper) takes place in the oocytes by means of numerous micropinocytotic coated pits of the oolemma. These invaginate and pinch off to form the intracellular, coated vesicles (Figs. 6, 7). Coated vesicles, with a diameter of 0.21 to 0.29 μm, gradually transform into round or somewhat irregularly shaped endosomes without coating. These endosomes may fuse to the limiting membrane of the primary yolk granules derived earlier from Golgi complexes and apparently also with each other to form large, membrane-bound transitional yolk bodies with loosely-packed contents of moderate electron density (Fig. 6). As the maturation of the yolk bodies proceeds, these are characterized by the presence of the centrally located electron-dense cores that exhibit a crystalline organization (Figs. 6–8). The mature yolk bodies become extremely large, up to 7–10 μm in diameter, although they are variable in size.

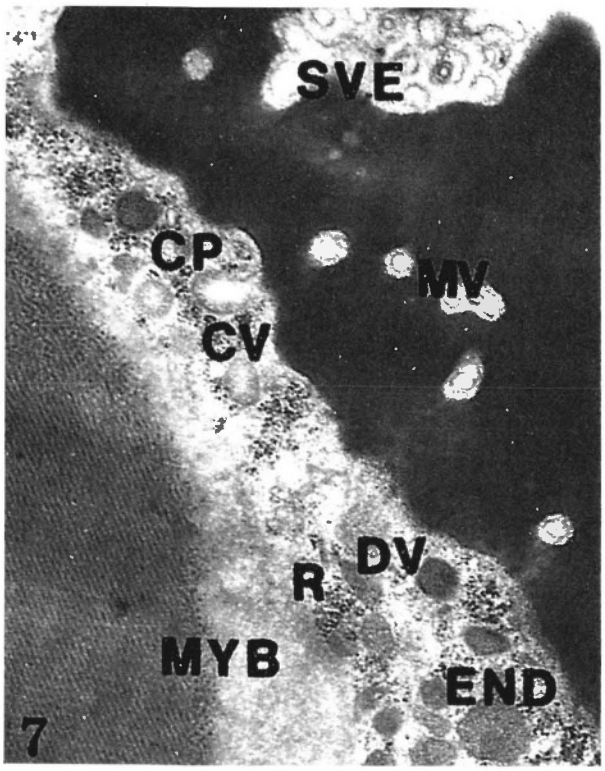
In addition to the formation of membrane-limited yolk bodies, lipid droplets are deposited in the ooplasm during the course of vitellogenesis (Fig. 8). In the fully-grown oocytes inclusions of these two types are represented in approximately equal proportion and gradually fill most of the ooplasm. Multivesiculate bodies, which have been observed in the ooplasm of some arachnids, including ticks (HECKER & AESCHLIMANN, 1970; OSAKI, 1971; BALASHOV, 1979), may sometimes be seen predominantly in the peripheral cytoplasm of the growing oocytes of *H. zachvatkini*. They presumably originate from the modified mitochondria or Golgi complexes, as has been shown for females of the argasid tick *Argas (Persicargas) arboreus* (EL SHOURA *et al.*, 1989). Unlike the latter, multivesiculate bodies in trombiculid oocytes seem to appear only at the late stages of vitellogenesis and are apparently never involved in the formation of the yolk bodies.

The remaining ooplasm between the packed yolk bodies and lipid droplets contains free ribosomes and some round electron-lucent mitochondria often with partially reduced cristae. At the end of vitellogenesis, the cortical vesicles disappear, and the oolemma becomes smooth and tightly opposed to the electron-dense secondary vitelline envelope at its outer and to the yolk spheres and lipid droplets at the inner surface.

During the course of the great cytoplasmic growth and oocyte migration to the periphery of the ovaries, somatic cells follow the oocytes with long extensions







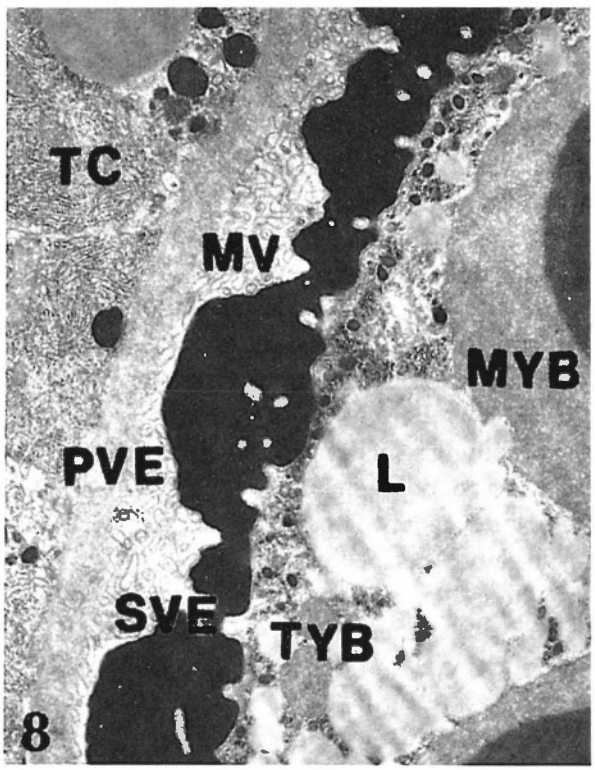
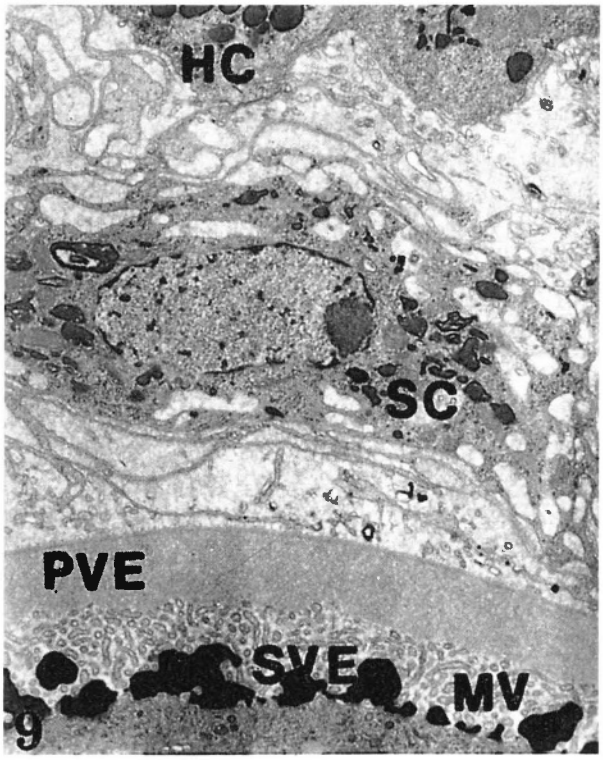


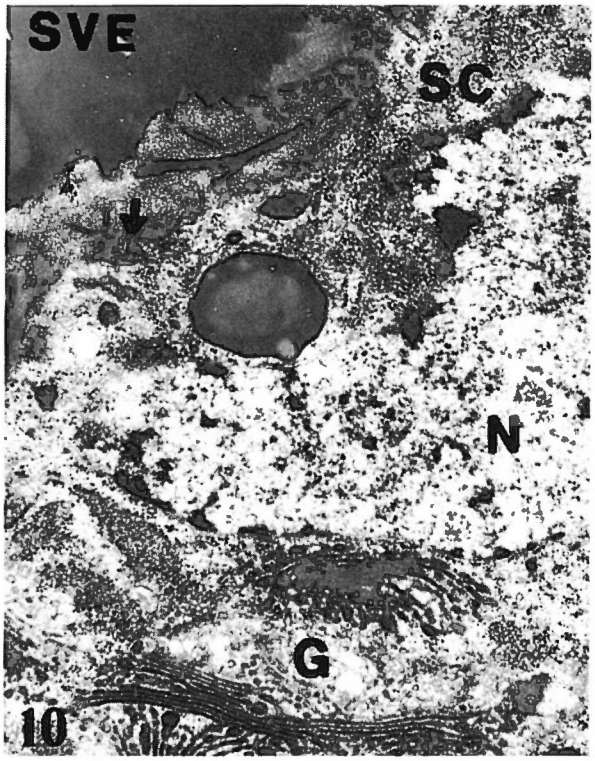
FIG 5: Cortical zone of oocyte at the early stage of vitellogenesis, situated in the ventral part of the ovary in close contact with haemocoelic space (HS) near the integument. Note the thin basal membrane of the ovary (BM), together with the primary vitelline envelope (PVE). G, Golgi complex, L, lipid inclusions, M, mitochondria, MV, microvilli, SVE, electron-dense patches of the secondary vitelline envelope, TYB, transitional yolk body. × 21 500.

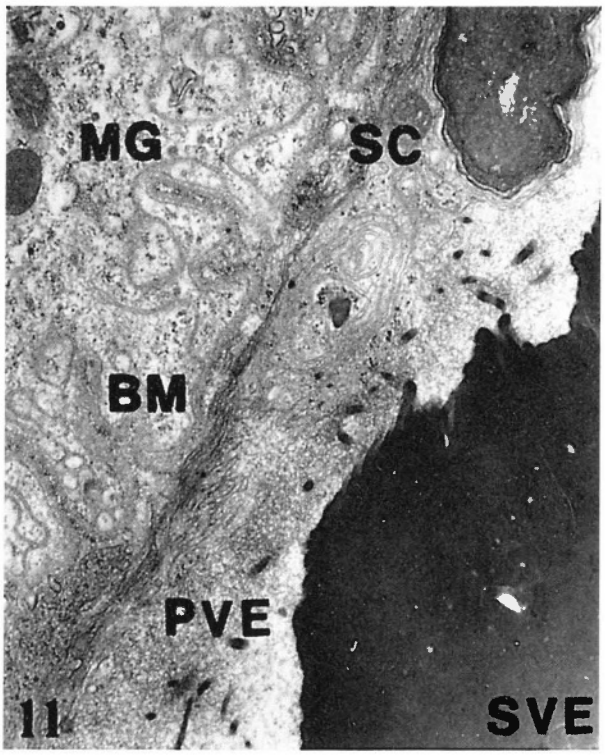
FIG 6: Part of more advanced oocyte, with primary (PVE) and secondary (SVE) vitelline envelopes, endosome (END), transitional (TYB) and mature (MYB) yolk bodies, and cortical ooplasm filled with coated pits (CP) and dense (DV) vesicles. R, ribosomes. Other abbreviations as in Fig. 5. × 16 000.

FIG 7: As in Fig. 6, but higher magnification of cortical zone, showing coated pit (CP), vesicle (CV) and endosome (END). Note the crystalline structure of the core of a mature yolk body (MYB) and microvilli (MV) in the pore canals of the secondary vitelline envelope (SVE). Other abbreviations as in Fig. 6. × 29 500.

FIG 8: Peripheral part of oocyte, located inside the ovary near the trophic cells (TC), with primary (PVE) and secondary (SVE) vitelline envelopes. Other abbreviations as in Figs. 5–7. × 8 500.







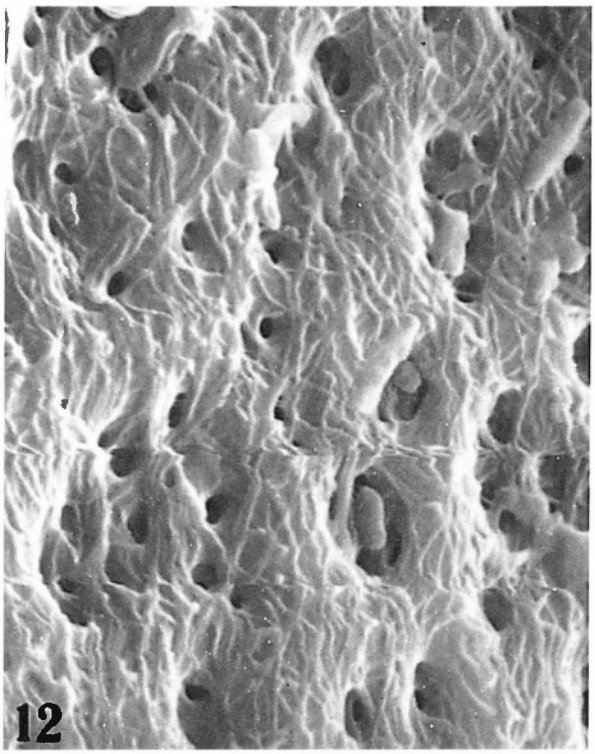


FIG 9: Somatic cell (SC) following oocyte, situated in close proximity to the integument without basal membrane of the ovary. HC, haemocyte of the haemocoelic space, MV, microvilli, PVE, primary and SVE, secondary vitelline envelope. × 7 500.

Fig 10: Formation of the external layer of a secondary vitelline envelope (SVE) through high secretory activity (arrow) of somatic cell (SC), provided with prominent Golgi complexes (G). N, nucleus. × 16 000.

FIG 11: As in Fig. 10, but showing restored basal membrane of the ovary (BM). MG, midgut, PVE, substance of a primary vitelline envelope. Other abbreviations as in Fig. 10. × 19 000.

Fig 12: Surface of the laid egg with numerous pores (SEM). × 15 000.

(FIG. 9) and partially or entirely encircle them towards the end of vitellogenesis. Furthermore, the basal membrane of the ovary (*tunica propria*) is often seen restored (Fig. 11). Owing to high secretory activity of somatic cells, largely due to the functioning of the well developed Golgi complexes (Fig. 10), electron-dense vesicles are secreted into the space around the oocyte occupied by the loosely-packed material of the primary vitelline envelope. The substances produced by the somatic cells in the form of electron-dense, often elongate drop-like particles, penetrate the primary vitelline envelope, presumably by as yet unknown physicochemical gradients. Finally, they are associated with the secondary vitelline envelope to form its external layer (Figs. 10, 11). The latter is not clearly distinguished from the underlying electron-dense layer of the envelope. At the end of this process, oogenesis is completely finished. The deposited mature eggs possess only a homogeneous secondary vitelline envelope (egg shell), of high electron density, whose fine superficial structure is clearly seen in the SEM (Fig. 12). Its maximum thickness in oocytes of *H. zachvatkini* varies from 1.6 to 3.3 μm.

The apical plexus of microvilli and solitary microvilli, located in the pore canals of the secondary vitelline envelope, is often completely destroyed before the external layer of the egg-shell is formed. The primary vitelline envelope has a similar destiny, although the residues of this envelope can occasionally be seen in some parts of the ovaries without oocytes and mature eggs.

It is a characteristic of trombiculid oocytes, in contrast to other arthropods with an analogous type of oogenesis, that the pore canals in the secondary vitelline envelope do not close entirely, even after the eggs have been laid, which may facilitate the respiration of the embryo. Moreover, during the formation of the secondary vitelline envelope, the site of oocyte attachment to the somatic cells gradually loses its precise function and disappears towards the end of vitellogenesis. This is why, in the envelope of deposited *H. zachvatkini* eggs, contrary to erythraeid mites (WITTE, 1975) and gamasids (WITALINSKI, 1986, 1987), no specialized regions, such as a micropyle-like structure, have been observed.

DISCUSSION

The present study indicates that the process of oogenesis in trombiculid mites is similar to that in ixodid ticks (AESCHLIMANN & HECKER, 1969; HECKER & AESCHLIMANN, 1970; BALASHOV, 1979; EL SHOURA *et al.*, 1989) as well as that in spiders of the families Ctenidae and Agelenidae (MOTHES & SEITZ, 1980). In these arachnids, during the previtellogenic period, the endogenous yolk precursors are synthesized by the cell organelles, such as Golgi complexes or the endoplasmic reticulum, in addition to the formation of surface microvilli. During vitellogenesis, or great cytoplasmic growth, proteins derived from exogenous sources, such as the mid-gut or fat bodies (COONS *et al.*, 1982), subsequently pass through the haemocoel to the ovaries. Finally, they enter the oocyte from the perivitelline space via coated pits and vesicles, the universal structures for specific endocytosis (ROTH & PORTER, 1964; RAIKCHEL & DHADIALLA, 1992). This process leads to the formation of the main yolk masses. The same process occurs in a horseshoe crab, *L. polyphemus* (DUMONT & ANDERSON, 1967). These findings show that vitellogenesis in trombiculids is practically affected by extraoocytic factors (the exogenic mode of vitellogenesis).

The unique primary vitelline envelope of trombiculid mites cannot be considered anatomically as a basal membrane (*tunica propria*), according to various characteristics, although, to some extent, it might be expected to play an analogous role. No other such instances of primary vitelline envelope deposition have been reported in detail in other animals. The significance of such a microvillar contribution to the formation of the primary envelope is a rather unusual phenomenon, and it offers an interesting comparison to other types of basal membrane and oocyte envelope formations.

The simultaneous formation of the vitelline envelope, the so-called secondary vitelline envelope of trombiculid mites, and the principal yolk masses of the oocytes is very characteristic for acarines (HECKER & AESCHLIMANN, 1970; ALBERTI, 1974; BALASHOV, 1979; WITALINSKI, 1986, 1988; EL SHOURA *et al.*, 1989) and other arachnids (OSAKI, 1971, 1972; MOTHES & SEITZ, 1980; WITALINSKI & ZUWALA, 1981) as well as for Xiphosura (DUMONT & ANDERSON, 1967). These animals demonstrate the various

modes of the solitary type of oogenesis, reflected in the endogenous formation of the envelope. The material of the vitelline envelope of *H. zachvatkini* oocytes seems to be produced with the participation of the Golgi complex, as indicated by the accumulation of Golgi-derived electron-dense vesicles in the cortical ooplasm. Vesicles deliver their contents into the perivitelline space between the bases of microvilli, which can be considered as the typical process of egg-shell formation in most arachnids with the solitary type of oogenesis, including trombidiform mites (AESCHLIMANN & HECKER, 1969; OSAKI, 1972; BALASHOV, 1979; MOTHES & SEITZ, 1981; WITALINSKI, 1986, 1987, 1988). There are, however, some variations in this process among arachnids. In the spider *Heptathela kimurai* the vitelline envelope, termed chorion by OSAKI (1971), is light-staining. In a harvestman, *Leiobunum* sp., the vitelline envelope, as shown by REGER (1970), arises from Golgi-derived vesicles of low electron-density.

Large numbers of vesicles, both exocytotic and endocytotic in nature, in a cortical cytoplasm of vitellogenic oocytes is a quite characteristic feature not only for arachnids (HECKER & AESCHLIMANN, 1970; BALASHOV, 1979; KESSEL & BEAMS, 1980; WITALINSKI & ZUWALA, 1981), but also for other arthropods (BEAMS & KESSEL, 1963) in which oocytes develop without follicular cells, partially or entirely outside of the ovaries. The interpretation of vesicles is often difficult (BEAMS & KESSEL, 1963; WITALINSKI & ZUWALA, 1981) because the process of vitelline envelope deposition is simultaneous with the concomitant process of yolk accumulation.

Nevertheless, as had been shown for a tetranychid mite, *Tetranychus urticae* (FEIERTAG-KOPPEN and PIJNACKER, 1985), and a limnocharid mite, *Limnochares aquatica* (WITALINSKI, 1988), the formation of the electron-dense vitelline envelope begins after the onset of vitellogenesis. Another mode of oogenesis is known in an oribatid mite, *Hafenrefferia gilvipes* (Oribatida), the vitellogenic oocytes of which are surrounded by follicular cells and devoid of microvilli (WITALINSKI, 1986). These cells secrete the material of the vitelline envelope, accompanied by the process of endogenous (autosynthetic) vitellogenesis. This results, in particular, in the absence of pore canals in the vitelline envelope.

The most characteristic feature of egg-shell formation in *H. zachvatkini*, which distinguishes trombiculid mites from other arthropods so far studied, is the deposition of the external layer of the secondary vitelline envelope by somatic cells of the ovaries. These cells gradually surround the growing oocytes and may be considered, in this instance, as substitutes for the true follicular cells. Thus, REGER's question (1977, p. 123), whether or not vitelline envelope formation by both the oocyte and follicle cells occurs in species other than amphibians, obviously receives a positive answer. The precise phylogenetic significance of this structural peculiarity in trombiculid oogenesis is not clear because of the scarcity of data on other groups of mites, although it may be interpreted as a new phylogenetic acquisition. Such a hypothesis, however, needs verification by detailed comparative analyses.

It is important to note that no other subsidiary envelopes (chorion) are formed in the oviducts or uterus of trombiculid females. In latter, oviduct cells are organized to form an osmoregulatory barrier, though this subject is beyond the scope of the present paper. This finding again distinguishes trombiculid mites from all other investigated Actinedida (BEAMENT, 1951; LEES, 1961; MATHUR & LE ROUX, 1970; ALBERTI, 1974; WITTE, 1975; MOTHES & SEITZ, 1981; WITALINSKI, 1988) and Acaridida (HEINEMANN & HUGHES, 1970; MAZZINI & BAIOCCHI, 1983; CALLAINI & MAZZINI, 1984). In these acariform mites, special external envelopes of the eggs, namely the chorion, often with a complex structure, are formed in the oviducts and other components of the reproductive system. These are thought to facilitate several important functions of the embryo, particularly respiration and absorption (water and gas exchange) (CALLAINI & MAZZINI, 1984; WITALINSKI, 1988). A compound structure of the chorion had also been reported for ixodid ticks (EL GOHARY *et al.*, 1986).

A detailed account of vitelline envelope functions and the process of fertilization in trombiculid mites is beyond the scope of this paper, but this needs to be thoroughly investigated in further studies.

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