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Fine Structure of the Nucleus and Cytoplasmic Feulgen-Positive Areas in the Developing Oocyte of *Argus (Persicargas) radiatus*

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FINE STRUCTURE OF THE NUCLEUS AND CYTOPLASMIC FEULGEN-POSITIVE AREAS IN THE DEVELOPING OOCYTE OF *Argas (Persicargas) radiatus*

by

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ABSTRACT

FINE STRUCTURE OF THE NUCLEUS AND CYTOPLASMIC FEULGEN-POSITIVE AREAS IN THE DEVELOPING OOCYTE OF Argas (Persicargas) radiatus.

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A transmission electron microscope analysis of the changes that occur in the nuclear chromatin and the Feulgen-positive areas of the oocyte cytoplasm as the female develops, feeds and mates is described. The description includes analysis of oocytes from three types of females: unfed, unmated; fed, unmated; and fed, mated. In all types, dense material passes through the extremely porous nuclear membrane where it accumulates in the cytoplasm.

Nuclei of previtellogenic oocytes appear the same in all females studied. Fibrillar bodies and up to four large nucleoli are seen. No typical chromatin is seen, but structures resembling nucleosomes appear throughout the nucleus.

Nuclei of early vitellogenic oocytes have increased numbers of nucleosomes. At least one nucleolus is present and fibrillar bodies are seen.

Nuclei of late vitellogenic oocytes have lost almost all nucleosomes from the nucleus and the nucleoplasmic appearance is noticeably different from previously described stages. Only one large nucleolus is present and no fibrillar bodies are seen.

All pedicellar areas reveal no deposition of material resembling

chromatin that was seen as Feulgen-positive areas in histological sections. Extraction of DNA during processing may be the cause of the lack of chromatin found at the oocyte-pedicel interface.

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INTRODUCTION

In recent years much information has been reported about the process of oogenesis in tick species. These data are important in understanding the phenomenom of transovarial transmission of pathogens as well as regulation of the fertilization process applicable to biological control procedures.

Balashov (1968) describes oocyte growth from cytological studies of oogenesis of three tick species, Argas persicus, Hyalomma asiaticum and Ixodes ricinus. He found that during the period of greatest oocyte growth, which lasts until the first vitelline granules appear, the chromosomes gradually lose their Feulgen-positive staining properties as the primary oocyte progresses through diplonema. At these stages the chromosomes can no longer be seen in the nucleus and a nucleolus is present at this time. The lack of Feulgen-positive material in the nucleus continues through yolk formation and growth. By the end of yolk deposition the nucleus has shifted its position to the periphery of the oocyte, near the pedicel. The nucleolus and nuclear membrane dissolve and the bivalents form a karyosphere (Goroschenko, 1965) which is surrounded by a thin cytoplasmic zone and remains until fertilization.

In their report on the fine structure of oogonia and oocyte development in the ixodid tick, Dermacentor andersoni, Brinton and Oliver (1971b) note emissions of dense particulate material from the nucleus during oocyte development. The emissions appear to be in

response to a feeding stimulus and are similar in appearance to the material found within the vacuoles in the nucleolus.

Histological studies of oogenesis of the argasid tick, Argas radiatus (Proes, personal communication), show the Feulgen-positive areas appear to move out of the nucleus into the cytoplasm, where they are deposited at the periphery of the oocyte at the point where attachment to the pedicel occurs. Since this deposition is found in mated ticks only, it appears that the stimulus for the movement of the Feulgen-positive areas to the pedicelar area is associated with mating rather than feeding. For this reason, the Feulgen-positive deposition may have a male or a female origin.

The purpose of this study is to determine the changes that occur in the nuclear chromatin and the Feulgen-positive areas of the oocyte cytoplasm as the female develops, feeds and mates. The development stages to be observed are as follows: unfed, unmated; five days post-fed, unmated; and five days post-fed and mated. These findings are important for an understanding of the influence of feeding and mating on oocyte development in the argasid tick, Argas radiatus.

LITERATURE REVIEW

Ovarian Structure of Newly Molted Females

Balashov (1968) described the ovary of argasids as unpaired, short and thick, and in the form of a sac from which the oviducts extend. In an unfed female, it was oval in transverse section and surrounded by a tunica propria. The lumen was lined with epithelial cells and extended the length of the ovary. The epithelial cells had no defined boundaries and their nuclei were smaller than those of oocytes. Immature oocytes had large vesicular nuclei which were surrounded by a comparatively narrow, easily distinguishable cytoplasmic zone. Recently molted females exhibited oogonia and oocytes in various stages of development from primary oocytes formed from recent oogonial division to mature oocytes filled with yolk droplets. Oocytes associated with the posterior, ovarian arch surface appeared more advanced than those on the anterior surface. Those on the anterior arch surface were mixed with oogonial cells.

Brinton and Oliver (1971a) studied ovarian development in the ixodid tick, Dermacentor andersoni. Oocytes, epithelial cells, pedicel cells, and muscle were the main components of the adult ovary. The ovary wall was comprised of epithelial cells and oocytes and surrounded a lumen which ran the length of the ovary in newly molted females. After a few weeks post-molting, unfed female ovaries developed a longitudinal groove along the entire length of the ovary causing the lumen, now completely lined with epithelial cells, to

collapse. Orientation of oocytes remained essentially unchanged.

Studies of Argas arboreus showed newly molted females had oocytes protruding into the hemocoel (Khalil, 1969). Primary oocytes had entered the "first growth phase" which corresponded to Balashov's stage I and II, during which time the nucleus underwent some changes. The nucleoplasm lost its basophilic nature and became finely granular. Fine chromatin threads protruded from the compact tetrads adhering to the nuclear membrane, the nucleolus enlarged, became vacuolated, and a small, round, darkly staining body appeared. Simultaneously, the oocyte penetrated the epithelium and came to lie under the tunica propria. The layer of epithelial cells under each oocyte bulged to the outside forming a pedicel with a central lumen to which the oocyte was attached.

Studies on Hyalomma excavatum (Khalil, 1970) showed oogonia and primary oocytes in less advanced stages occupying the inner wall of the ovary. Those primary oocytes with their nuclei in a nondescript phase (Khalil's "interphase") were slightly larger in size and occupied the outer portion of the ovarian walls. None of the oocytes had protruded above the ovary surface yet as in A. arboreus (Khalil, 1969). The oogonial nuclei contained two basophilic nucleoli but otherwise was similar in appearance to oogonia of A. arboreus. Primary oocyte nuclei resembled those of oogonial cells but usually contained only one nucleolus. Primary oocyte nuclei in "interphase" had tetrads that at first were basophilic and adhered to the nuclear membrane, but gradually lost their basophilic nature and finally disappeared. One nucleolus was present; the nucleoplasm was coarsely granular and there was a fine chromatin network in "interphase"

nuclei. The "first growth phase" did not occur until after the adult fed.

The ovary of the unfed, virgin female of Haemaphysalis longicornis was similar in appearance to H. excavatum (Khalil, 1972). Oogonia and recently differentiated primary oocyte nuclei were also similar to H. excavatum although primary oocyte nuclei contained two nucleoli. The "interphase" nuclei of primary oocytes had chromosomes adhering to the nuclear membrane and two basophilic nucleoli; the nucleoplasm did not stain with hematoxylin or eosin. No further growth occurred until feeding began.

Fine structure studies of D. andersoni were carried out by Brinton and Oliver (1971b). In newly molted adults, oocytes and their nuclei were found to be elliptical to round, each with irregular margins and "dimensional variability". These also exhibited high nuclear-cytoplasmic ratios at this stage of development. The organization of some nuclear material in this stage suggested nucleolar formation. The nuclear membrane possessed distinct pores and through these, dense particulate material was seen migrating to the ooplasm. Dense granular material of varying particulate size was interspersed among mitochondria.

In the five month post-molt adult, they found oocytes in the longitudinal groove to be about 10 μm smaller than oocytes around the rest of the ovary. Cell membranes were irregular but explicitly defined and mitochondria were present throughout the cytoplasm. The cytoplasm appeared granular because of an even distribution of ribosomes that were not associated with endoplasmic reticulum. The nuclei had irregular margins with distinct pores, granular nucleoplasm with

electron-dense granules of varying size, and a distinct nucleolus that was usually vacuolate. In some cases, electron-dense satellite bodies were seen "budding" from nucleoli. Within vacuoles of the nucleolus, a higher density of granulate material was present than that found in other regions of the nucleoplasm. Similar dense material migrated to the nuclear membrane where it accumulated prior to passage into the ooplasm.

Structure of the Ovary of Fed, Virgin Females

A bloodmeal was necessary for further ovarian growth and would stimulate development through Balashov's (1968) stages II, III, and IV in the three species studied. Stage II marked the beginning of great growth and lasted until the appearance of the first vitelline granules in the oocyte cytoplasm. The nucleolus was spherical, relatively homogeneous, and enlarged rapidly during this stage. The bivalents formed during stage I rapidly dissociated and disappeared; optically the nucleus appeared empty and lacked positive Feulgen staining bodies. The oocytes now protruded above the ovary surface. Primary yolk inclusions appearing along the peripheral cytoplasmic zone marked the beginning of stage III. Yolk deposition was dependent on nutrients from the blood meal (Oliver, 1974). Oocytes, nuclei, and nucleoli all increased in size. As nuclei reached maximum size, lateral lobes and protrusions were formed. Vacuoles appeared within the nucleolus; small fragments were sometimes shed from the nucleolus and were not identifiable in the nucleoplasm. No DNA was apparent within the nucleus. The oocyte began formation of a dense homogeneous, double-membraned vitelline membrane. Stage III ended and stage IV began when large yolk droplets filled the oocyte and yolk deposition

ended. The nucleus was not irregularly angular and had shifted to the oocyte periphery. Oocytes ended growth at this stage and bulged into the body cavity, connected to the ovary wall by a pedicel of epithelial cells. No further growth occurred until fertilization (Balashov, 1968).

Ovarian development in D. andersoni also resumed with a bloodmeal. Forty-eight hours after attachment of the female to the host, distinct changes were noted in size and form of the ovary. Oocytes, except those in the longitudinal groove, moved to a position above the ovary where they protruded into the hemocoel, attached to the ovarian wall by a pedicel of epithelial cells. There was a cessation of visible ovarian maturation at this point (Brinton and Oliver, 1971a).

Oocytes in A. arboreus remained in a condition similar to those in the ovary of unfed, inseminated females, but the ovary contained a greater number of oocytes. Oocytes were at the same stage of development as for unfed, unmated females (Khalil, 1969).

Feeding H. excavatum females exhibited large ovarian size increases and primary oocytes protruded slightly on the surface after three to five days. Three weeks following attachment the unmated female's ovary size did not exceed that of mated females attached for five days. Only a few oocytes reached the early vitellogenic stage, the rest of them were previtellogenic. During this growth phase, the nucleoplasm became finely granular, the nucleoli enlarged and became vacuolated while the growing oocytes protruded into the hemocoel to lie under the basement membrane. The epithelium below the oocytes formed a pedicel with a central lumen to which the oocyte was attached (Khalil, 1970).

In parthenogenetic H. longicornis females that had fed for three to four days, the ovary enlarged greatly and oocytes protruded from its surface into the hemocoel. The ovarian inner fold remained until the tick detached from the host. The first growth phase of oocytes began while the female fed. The nucleoplasm became finely granular and eosinophilic; the nuclei and the nucleoli enlarged, the nucleoli became vacuolated; and the chromosomal tetrads gradually lost their basophilic appearance and disappeared. The epithelium below the oocytes formed a pedicel to which the oocyte was attached. The nucleus of the oocyte migrated toward the pedicel cells but it did not become diffused as it did in a later developmental stage in H. excavatum (Khalil, 1972).

Fine structure studies by Brinton and Oliver (1971b) on D. andersoni showed that 48 hours after attachment to the host, oocytes in the longitudinal groove and around the periphery of the ovary had enlarged. Those oocytes within the longitudinal groove still maintained a high nuclear-cytoplasmic ratio. The ooplasm was densely granular, and the nucleoplasm had a mosaic appearance. In light microscope sections, the nucleoplasm was reticulated and frequently contained two nucleoli. Intracellular morphology of oocytes around the ovary periphery was similar to that of longitudinal groove oocytes, but number and activity of organelles present was quantitatively different. Small groups of developing microvilli projected from the oolemma and bent in different directions forming an irregular brush border abutting on the tunica propria.

The nucleoplasm contained many granules of varying size and shapes, some in close proximity to the nuclear membrane. Chromatin or

nucleolar material was clumped in both large and small units forming a mosaic pattern. Emission of dense material via highly porous nuclear membranes was frequently observed, and resulted in formation of extranuclear granular bodies resembling nucleoli in structure. Mitochondria were often intimately associated with and partially fused to such emissions (Brinton and Oliver, 1971b).

Ninety-six hours after attachment, oocytes within the longitudinal groove had undergone some enlargement. Ribosomal bodies were abundant in the ooplasm and nucleoli, sometimes paired, were prominent. "Blebs" from the main nucleolar mass underwent additional budding to form smaller tertiary satellite bodies. Electron-dense material, very similar in form and density to these satellites, eventually passed through pores in the nuclear membrane. Densely stained material was in the cytoplasm immediately adjacent to the periphery of the nucleus. Ribosomal particles continued to be emitted from the nuclei of oocytes not in the longitudinal groove (Brinton and Oliver, 1971b).

Structure of the Ovary of Fed, Mated Females

Khalil (1969) reported that oocytes in fed, mated females of A. arboreus underwent a "second growth phase", which included vitellogenesis described by Balashov's stages III and IV. Similar growth occurred in oocytes in fed, mated females of H. excavatum (Khalil, 1970). In parthenogenetic H. longicornis, the second growth phase began one day after the tick detached from the host; no mating was necessary for development and ovulation (Khalil, 1972).

Oocyte maturation in D. andersoni was greatly accelerated after mating and completing engorgement. Twenty-four hours after completing engorgement, oocytes were of nearly constant size, except for those

in and near the longitudinal groove where they were about half size. Forty-eight hours after engorgement, longitudinal groove oocytes underwent some enlargement. Seventy-two hours after engorgement, oocyte development proceeded at different rates with the largest oocytes containing numerous vitelline spheres (Brinton and Oliver, 1971a).

Transition zones were present in pedicel cells having advanced oocytes attached and micropyles were seen in the cuticular walls at sites of transition zones. Transition zones were essentially continuous from sites of oocyte attachment through pedicel and epithelial cells to channels formed by the lumenal epithelium. A transition zone consisted of an intercellular zone which had become altered to form a channel which was in direct line of communication with the micropyle of oocytes (Brinton, Burgdorfer and Oliver, 1974).

Transmission electron microscopy studies of fed, mated D. andersoni females revealed that from six days after attachment to 24 hours after completing engorgement, oocytes outside the longitudinal groove had a distinct irregular brush border of microvilli. Many oocyte microvilli were in intimate contact with microvilli from pedicel cell membranes. Amorphous material similar to that around the remaining periphery of oocytes and in micropinocytotic pits and tubes was also present in these areas. Vitellogenesis proceeded concurrently with elaboration of cuticle. Patches of densely stained material were still present adjacent to the nuclear membrane prior to their emission into the cytoplasm. Dense masses of material emitted from the nucleus often assembled around yolk spheres (Brinton and Oliver, 1971b).

Forty-eight hours after detachment, advanced oocytes had increased in diameter and, without exception, nuclei of oocytes were located in

the basal region adjacent to that area where the cuticle attached to pedicel cells. The nucleoplasm was coarsely granular and nucleoli were still present. Prominent pores were evident in the nuclear membrane. Each pore exhibited a fine membrane across the diameter which appeared to act like a sieve, since after passage through the pores, the dense and concentrated material of the nucleoplasm became more diffused. The nucleolus was prominently reticulated (Brinton and Oliver, 1971b).

MATERIALS AND METHODS

Ticks

A laboratory stock of A. radiatus received originally from Dr. James Oliver, Georgia Southern College, was fed on chickens and maintained between feedings at 25C, 85% R.H. and a 12 hour light cycle. All nine ticks selected for the experiment were newly molted, unfed, virgin females. Three were held without feeding or mating. Five days before fixation, six of the above females were fed. Three of the fed females were mated after feeding. Mating was confirmed by observance of the external spermatophore over the genital pore of the female.

Fixation and Embedding

The overies were dissected from all nine ticks and fixed in 4% glutaraldehyde in 0.1M s-collidine buffer (pH 7.4) at 0 C for two hours. Tissue was washed in two changes of 0.2M s-collidine buffer for 15 minutes. Post-fixation followed with 0.1M s-collidine buffered (pH 7.4) 1% osmium tetroxide for one hour at room temperature. Ovaries were washed twice with 0.2M s-collidine buffer and dehydrated at room temperature through an ethanol series (five minutes in each solution) followed by two ten-minute changes of 100% ethanol. Two 30 minute changes of propylene oxide preceded infiltration in 1:1 propylene oxide: Epon 812 for 24 hours and pure Epon 812 for one hour. Tissue was embedded in Epon at 60 C, 15 psi for 24 hours.

Sectioning, Staining and Examining

Most sections were cut with glass knives on a LKB 8800 Ultratome III ultramicrotome; a few were cut with a DuPont diamond knife using the same microtome. Thick sections (1μ) were transferred with a wire loop to glass slides and stained with a solution containing 1% each of Azure II, sodium borate and methylene blue. Thin sections (60Å to 150Å) were picked up on 300 mesh copper grids and stained for 15 minutes in a saturated solution of urinyl acetate in 50% ethanol and then for one to two minutes in lead citrate solution. Grids were examined on a Philips 301 transmission electron microscope. At least ten eggs from each of two females were examined in each category. Only one vitellogenic oocyte was sectioned in the fed, unmated category, but others were present.

RESULTS

Three stages of oocyte development were identified in the study: previtellogenic (PV) in which the oocytes had no yolk formation, early vitellogenic (EV) in which the oocytes had yolk droplets in early stages of development and late vitellogenic (LV) in which the oocytes were filled with yolk droplets. The only stage seen in the unfed, unmated females (UU) was PV. All three stages were seen in the fed, unmated (FU) and fed, mated (FM) females. Comparisons were made not only between different types of females, but also between oocytes at the same stage of development in each type of female. All oocytes studied were protruding above the ovary wall and were attached by a pedicel. All tick cell nuclear membranes observed were extremely porous which agrees with all previous reports of the structure (Figs. 1, 2, 10, 11, and 12).

Nucleus - PV Oocytes

Nuclei of PV oocytes appeared the same in all types of females. The nucleoplasm had a fine fibrillar matrix with granules of varying sizes interspersed (Fig. 2). The granules were denser in some areas within the nucleus and could be seen in light microscope sections as medium, light stained areas (Fig. 3). Dense material could be seen passing through the pores and accumulating outside the nuclear membrane (Figs. 2, 4).

A well defined nucleolus was the most prominent nuclear organelle,

and as many as four large nucleoli and several "buds" might be found in one nucleus. Nucleoli were composed of fibrillar and granular components and were variably vacuolated (Figs. 1, 5). A denser fibrillar matrix was found within the vacuoles of the nucleolus in contrast to that found in the nucleoplasm (Figs. 2, 5). Some granules were seen in the vacuolar areas of the nucleolus. The non-vacuolar areas within the nucleolus were variable in staining and this differentiation could be detected in both light and electron photomicrographs (Fig. 1). PV oocytic nuclei contained fibrillar bodies which varied in size and number (Fig. 6). These fibrillar bodies were similar to the secondary body (or corpusle) described by Aeschlimann and Hecker (1967) on the tick, Ornithodorus moubata. The fibrillar body diameter was much smaller than the large nucleoli, but it was comparable to that of the bud structures. Fibrillar bodies were well defined and composed of dense, coarse fibers with few granules interspersed.

At no time was typical chromatin material seen in oocytes. However, structures that resemble nucleosomes appeared in small clusters throughout the nucleus and along the nuclear membrane (Fig. 2). Since these structures were the same size and had the same appearance as nucleosomes, they will be referred to as such. These clusters also appeared as medium light stained areas in light microscope sections (Fig. 3). Some of the granules in the nucleoplasm appeared to be of nucleolar origin due to their size (Fig. 2). They were easily distinguishable from the nucleosomes at the electron microscope level, but they could not be differentiated from these at the light microscope level.

Nucleus - EV Oocytes

The nucleoplasm consisted of a fine fibrillar matrix with many granules of varying sizes, some of which appeared to be of nucleolar origin and others appeared to be nucleosomes (Fig. 7). EV oocytes appeared to have more granules and clusters than the PV oocytes (Figs. 8, 9). Dense material continued to pass through the porous nuclear membrane (Fig. 7) where it formed perinuclear bodies that resembled nucleoli in their staining properties at the light microscope level (Fig. 9).

A well defined, vacuolated nucleolus was again the most prominent nuclear organelle. A nucleolus was not seen in this stage in the FU females, but it was presumed to be similar in appearance and characteristics to the FM nucleolus because nucleolar characteristics were similar in all types of females studied. All nuclei contained at least one large nucleolus with small "buds". The material within the vacuoles was as described for the PV oocyte nucleus, but with fewer granules (Fig. 10). Fibrillar bodies were seen and appeared as described for PV oocyte nuclei.

Nucleus - LV Oocytes

The nucleoplasmic fibrillar matrix appeared more dense in both the FU and FM females than at an earlier stage of development of oocytes in the same types of females. The granules comprising the non-fibrillar part were almost exclusively of the size attributable to nucleolar origin. Few nucleosomes could be seen but those that were identifiable were scattered throughout the nucleoplasm (Fig. 11). The medium-light stained areas referred to in Figure 3 were seen, but in fewer numbers than in PV and EV oocytes (Fig. 13). The appearance of

the nucleoplasm was noticeably different and stained lighter in thick sections at this stage of development (Figs. 13, 14). Dense material continued to leave the nucleoplasm through the nuclear membrane pores, forming perinuclear bodies (Fig. 12).

In FM females only one large, well-defined, porous nucleolus was present in each nucleus, although a few, very small "buds" were usually evident. In FU females up to two large nucleoli were identifiable (Fig. 14).

The fibrillar component of the nucleolus was denser inside the vacuoles than in the nucleoplasm as mentioned previously (Fig. 11). Fibrillar bodies were not seen at the ultrastructural level in LV oocyte nuclei in either FU or FM females, although one or two small fibrillar bodies were seen at the light microscope level.

Pedicel - PV Oocytes

The pedicellar region of PV oocytes was very similar in all three types of females (Fig. 15). Microvilli extended from the oocyte cell membrane only and were restricted to the periphery of the oocyte-pedicel cell interface. The pedicel cells did not have microvilli at this stage. At some points of contact it was impossible to distinguish the two cell membranes separating oocyte from pedicel. No deposition of chromatin or chromatin-like material was seen.

Pedicel - EV Oocytes

At this stage the shell was being laid down around the oocyte and the number of free ribosomes in the cytoplasm had decreased in comparison to those seen in PV oocytes. Structures resembling polyribosomes were seen in the cytoplasm for the first time. Microvilli were

present and appeared to be of both oocyte and pedicel cell origin. No deposition of chromatin or chromatin-like material was seen (Fig. 16).

Pedicel - LV Oocytes

No deposition of any material resembling chromatin was seen along the attachment point of the FU females and in fact, little material was seen there and no yolk droplets were near (Figs. 14, 17). More material was seen along the periphery in the FM females, but was interpreted as dense cytoplasm displaced by developing yolk droplets (Figs. 13, 18). That material which was evident was found along the oocyte periphery at locations other than at the pedicel attachment point (Fig. 19). In areas where the material was not as concentrated (Fig. 20), yolk droplets were not located close to the oocyte periphery.

DISCUSSION AND CONCLUSIONS

The highly porous nuclear membrane seen in all cells in this study was reported previously for developing oocytes of the tick, D. andersoni (Brinton and Oliver, 1971b), the crayfish, Orconectes virilis (Kessel and Beams, 1968), and the snail, Ilyanassa obsoleta (Taylor and Anderson, 1969). The nucleoli at all stages in all categories had the same general organization and composition, similar to those of the tick D. andersoni (Brinton and Oliver, 1971b), A. arboreus (Khalil, 1969), H. excavatum (Khalil, 1970), and the crayfish O. virilis (Kessel and Beams, 1968). Two to four nucleoli and several "buds" were commonly found in the PV stage. This was consistant with reports of up to six large nucleoli per nucleus in A. radiatus and Argas sanchezi (Proes, personal communication). At least one large nucleolus and several buds were found in the EV stage. Khalil (1969), however, reported nuclei at these stages with only one nucleolus in A. arboreus. Oocytes that contained two or three nucleoli were rare, probably resulting from failure of cytoplasmic division following oogonial mitosis. In her report on H. excavatum (1970), Khalil noted that most nuclei contain one nucleolus. She found only two organisms in which all oocytes contain two and sometimes three subequal nucleoli. These were thought to represent an individual difference expressed only in a few ticks, but not an abnormal condition. Brinton and Oliver (1971b) reported one large nucleolus (occasionally two) and a few "buds" at all stages of development. In this study, LV oocytes of FM females were

observed to have one large nucleolus and a few very small "buds".

The fibrillar body might be associated with the mechanism for reduction of the number of nucleoli as the oocyte develops. Nuclear material, staining similarly to nucleoli, was seen passing through the porous nuclear envelope where it accumulated outside the nuclear membrane. This material passing through the nuclear envelope appeared to be composed of granular, or ribosomal, particles of the nucleoli. The fibrillar bodies might be what remains in the nucleus after this migration. The fact that no large fibrillar bodies were seen in LV oocyte nuclei supports this theory. A few very small fibrillar bodies were seen in light microscope sections conforming in size to the small nucleolar "bud" also seen at this developmental stage. A similar mechanism occurred in crayfish, Orconectes (Kessel and Beams, 1968), where many of the dense nucleoli in each oocyte nucleus underwent a partial or complete transformation into a network of coarse lamellae. After establishment of the new organization, the nucleoli decreased in size due in part to a progressive fragmentation from the ends of the granular lamellae. Many of the fragments were located in proximity to pores where frequent examples of nuclear-cytoplasmic exchanges could be demonstrated.

Preliminary studies found Feulgen-positive granules were present around the nuclear membrane only in oocytes that had not protruded above the ovary. PV oocytes protruding above the ovary had Feulgen-positive granules concentrated in the nucleolus with light staining along the inside of the nuclear membrane. At the start of or just before yolk deposition, Feulgen-positive granules left the nucleolus, and at about the time of heavy yolk deposition, they moved out of the

nucleus into the cytoplasm. Only in FM ticks were the Feulgen-positive granules located at the pedicellar area. As early as three days after mating Feulgen-positive granules could be seen at the pedicel and after four days post-mating, they were quite evident.

Others reported DNA loss from the nucleus, but did not find DNA appearing in the cytoplasm. Balashov (1968) reported that the chromosomes of Argas persicus lost their Feulgen-positive staining properties during stage II and the chromosomes did not reappear until the nucleolus and nuclear membrane dissolved and the bivalents formed a karyosphere. At no time in this study did the nucleolus and/or nuclear membrane dissolve. Khalil (1970) reported the tetrads formed in the oocytes of the tick H. excavatum, gradually lost their basophilic nature and disappeared, even before feeding and mating. In H. longicornis (Khalil, 1972) the tetrads lost their basophilic staining ability and disappeared after the female began feeding (during the "first growth phase").

Changes in the nucleoplasm at both the light and electron microscope levels followed the histological patterns of the preliminary studies. No typical looking chromatin was seen at any stage of development. Khalil (1969) had found compact tetrads adhering to the nuclear membrane after diakinesis in A. arboreus and fine chromatin threads protruded from them. Tetrads were also seen for a short time in primary oocytes of UU females of H. excavatum (Khalil, 1970) and H. longicornis (Khalil, 1972).

In this study structures resembling nucleosomes could be the Feulgen-positive granules seen in the histological study. Nothing comparable to nucleosomes in size could be detected in the nucleolus,

but the darker areas within the non-vacuolar areas could correspond to the Feulgen-positive areas seen in the preliminary studies in the PV stage. The appearance of more nucleosomes in the EV nucleoplasm could correspond with the loss of Feulgen-positive granules from the nucleolus at this stage of development. The changes in the LV stage nucleus at both the light and electron microscope levels indicated that some alteration had occurred. The loss of chromatin from the nucleosomes and eventual passage across the nuclear membrane might result in this changed staining pattern.

The darkly staining perinuclear material was found in oocytes before, during, and after vitellogenesis, and therefore, indicated that this material was probably not the Feulgen-positive areas seen in the preliminary studies. This conclusion was supported by the identification of similar perinuclear granules in D. andersoni (Brinton and Oliver, 1971b), O. virilis (Kessel and Beams, 1968), and I. obsoleta (Taylor and Anderson, 1969).

No deposition of material was seen in PV and EV oocytes and the pedicel cells and PV oocytes had extremely closely adhering membranes. The formation of the shell in EV oocytes provided a means of distinguishing oocyte from pedicel. Intimate contact of microvilli of oocytes and pedicel cells as reported by Brinton and Oliver (1971b) for D. andersoni was not seen.

The lack of any identifiable deposition of material at the LV oocyte periphery in the fed, mated category that could be identified tentatively as DNA was not supported by electron microscope studies. The preliminary studies showed a Feulgen-positive deposition three days post-mating in virtually every LV oocyte. Enough pedicullar

areas were observed in this study to reach the conclusion that the material seen in the photomicrographs was not DNA. Lack of a deposition of material could have resulted from extraction during processing. This was highly probable since attempts to stain thick sections with Schiff's reagent after Epon removal were unsuccessful.

No evidence from this study indicated whether the Feulgen-positive granules were of male or female origin. Meiosis was not observed at any stage of development and the nucleosomes could not be seen leaving the nucleus in an identifiable form. No sperm were seen in the ovarian lumen as reported by Khalil for H. excavatum (1970) and for A. arboreus (1969). Khalil (1969) contended that a Feulgen-positive, granulated field found in oocytes of A. arboreus during vitellogenesis resulted from the diffusion of the compact sperm nucleus in the oocytes. Wagner-Jevseenko (1958) stated that typical meiotic division in O. moubata did not occur, but instead that DNA was emitted from the nucleus and appeared as a Feulgen-positive granulated field. Roshdy (1961) considered this field to be groups of microorganisms. Microorganisms were observed in oocytes in this study, but these appeared in all categories and never appeared lining the oocyte periphery at the pedicellar attachment.

In conclusion, there was a definite relationship between feeding and mating and the growth of the oocyte. Oocytes from UU females never developed past the PV stage. Feeding stimulated vitellogenesis and growth and at five days post-attachment, a small number of oocytes were seen enlarged and with yolk. Mating provided the stimulus for yolk deposition in more PV oocytes as well as that needed for the completion of vitellogenesis in all oocytes.

Feeding and mating were also responsible for the changes that occurred in the nucleus. As oocytes matured through vitellogenesis, the number of nucleoli decreased from as many as four to one. Clearly the feeding and mating stimuli resulted in reduction of nucleoli number until only one remained in the mature LV oocyte. The fibrillar body was probably associated with the mechanism for reduction of the number of nucleoli. As with the nucleoli, the number of fibrillar bodies decreased as the oocyte developed through vitellogenesis until no fibrillar bodies were detected in LV oocytes.

A change in number of nucleosomes in the nucleoplasm as the oocyte matured was also evident. As the oocyte entered early vitellogenesis, there was an increase in the number of nucleosomes present in the nucleoplasm over that found in PV oocytes. Thereafter, commensurate with continued feeding and yolk deposition in LV cells, there was a reduction in the number of nucleosomes in the nucleus and a change in staining of the nucleoplasm. Mating did not appear to effect the movement of nucleosomes from the nucleus since LV cells appeared the same in both FU and FM females.

Electron dense granular material seen passing from the nucleus at all stages of development was composed of granular material equal in diameter to ribosomes. At the EV stage, ribosomes began congregating into polyribosomes, apparently initiated by the feeding process in the FU female.

As yolk was formed, cytoplasm was displaced peripherally and was compressed against the sides of the oocyte. No differences were seen in any of the peripherally displaced cytoplasm of LV oocytes, including that immediately adjacent to the pedicel. Therefore, the effect of

feeding and/or mating on the deposition of Feulgen-positive material at the oocyte-pedicel interface remained unknown.

Further work is needed to answer the questions about meiosis, chromosome dimunition, deposition at the pedicellar region and fertilization. A method is needed to enable the worker to process the tissue for plastic embedding and to remove the plastic from the one micron sections in such a way that Feulgen preparations for light microscopy can be accomplished. Light and electron microscope autoradiography is the next logical step to identify whether the Feulgen-positive deposition at the pedicel area after mating is of male or female origin.

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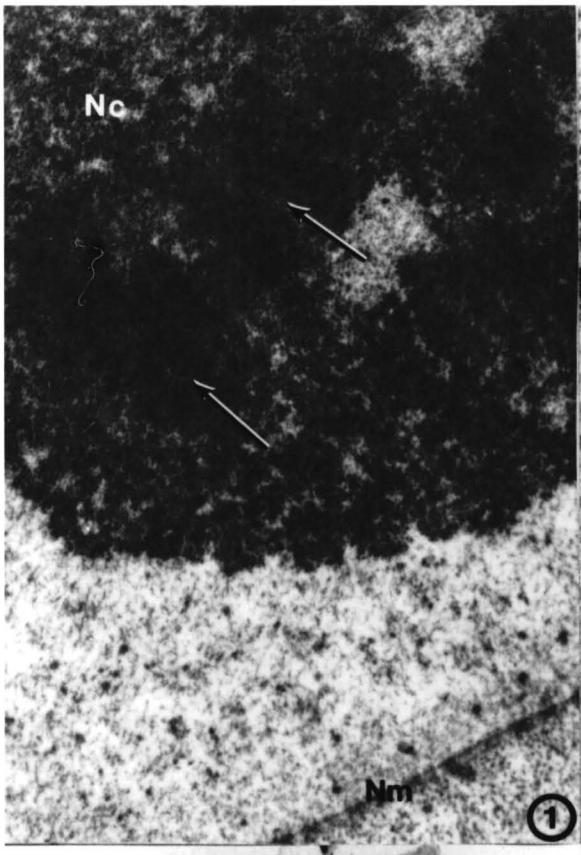
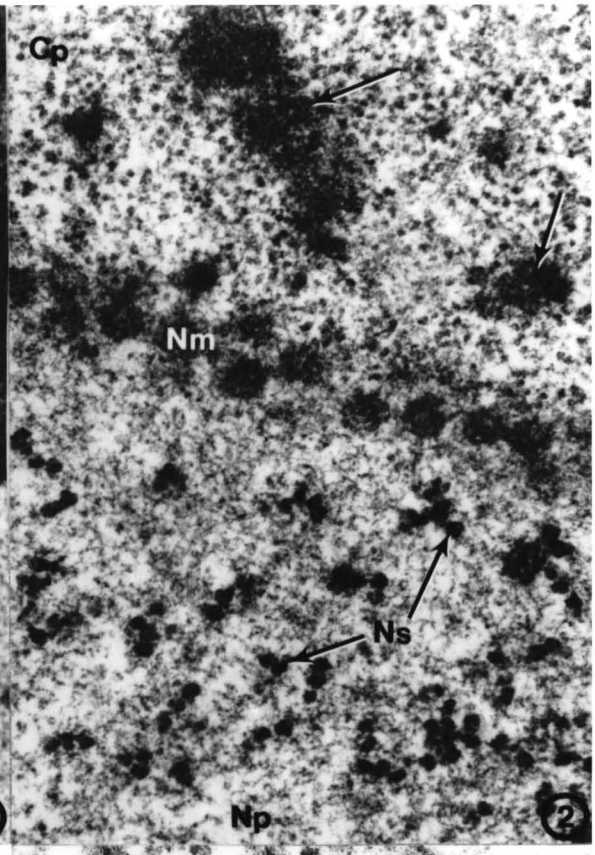
Fig. 1. Previtellogenic (PV) oocyte nucleus. Note extremely porous nuclear membrane and well-defined, vacuolated nucleolus with dark staining areas (arrows). Nc= nucleolus, Nm= nuclear membrane. x21,300.

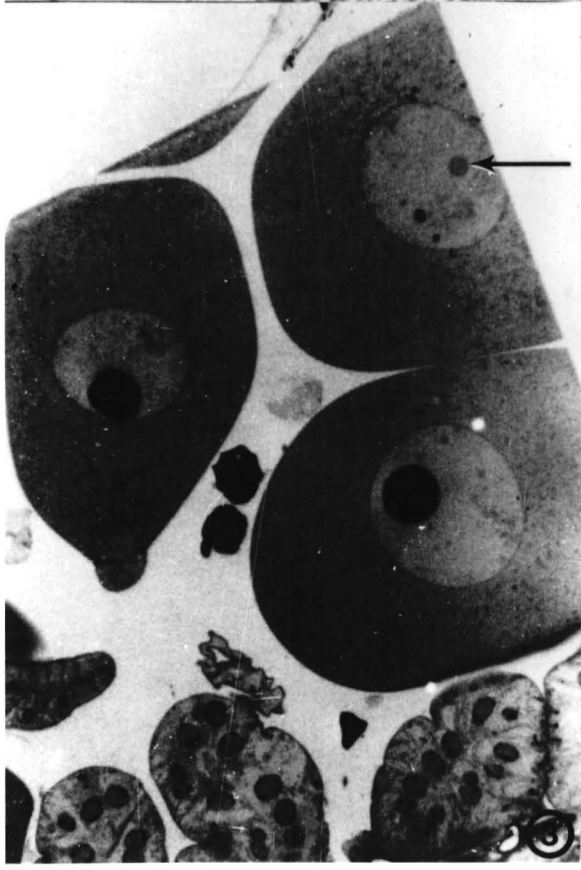
Fig. 2. Previtellogenic (PV) oocyte. Nucleoplasm shows granules of different size, nucleosomes (Ns) and small particles of nucleolar origin. Dense material (arrow) accumulates outside of nuclear membrane in cytoplasm. Np= nucleoplasm, Ns= nucleosomes, Nm= nuclear membrane, Cp= cytoplasm. x45,000.

Fig. 3. Previtellogenic (PV) oocyte, light photomicrograph. Note clusters of granules and fibrillar body (arrow) in nucleus. x160.

Fig. 4. Previtellogenic (PV) oocyte. Dense material is seen passing through nuclear membrane pore. Np= nucleoplasm, Nm= nuclear membrane, Cp= cytoplasm. x135,000.


Nc
Nm
1


Cp
Nm
Ns
Np
2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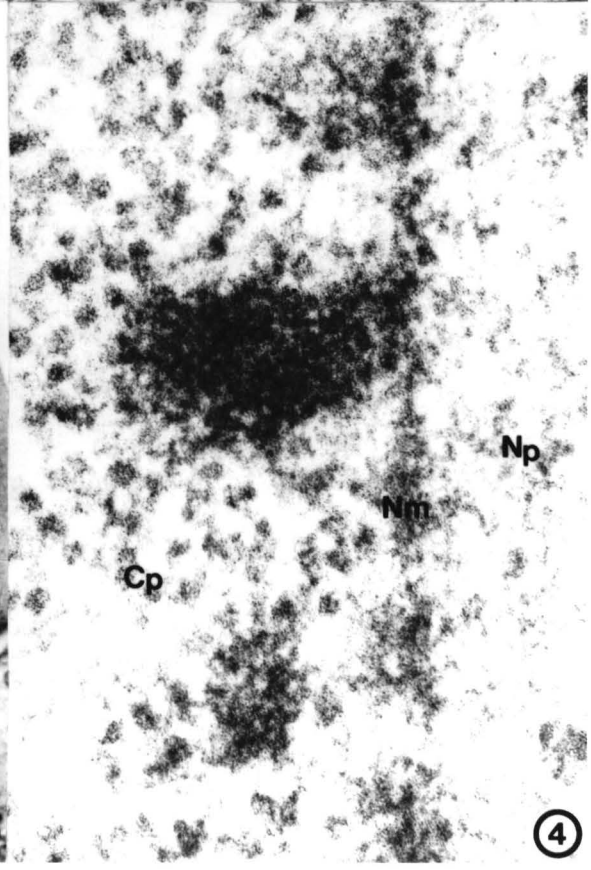
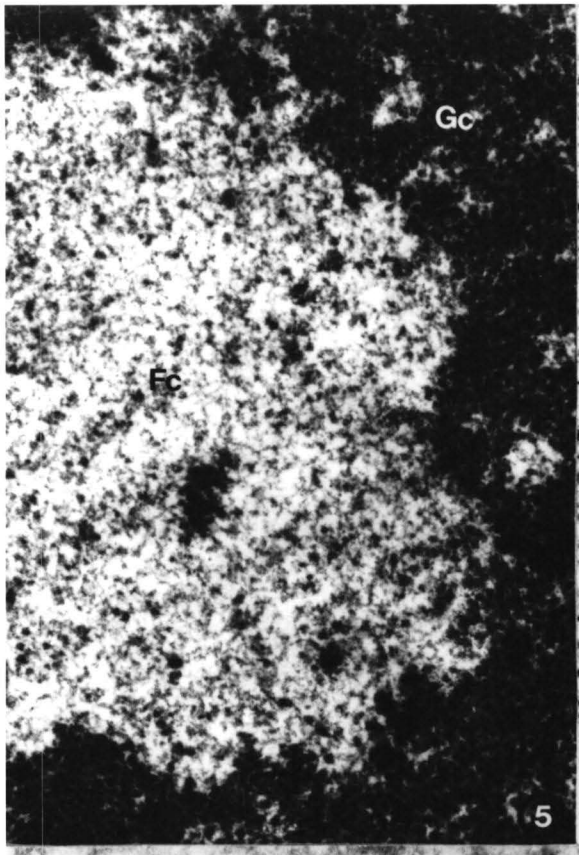

Np
Nm
Cp
4

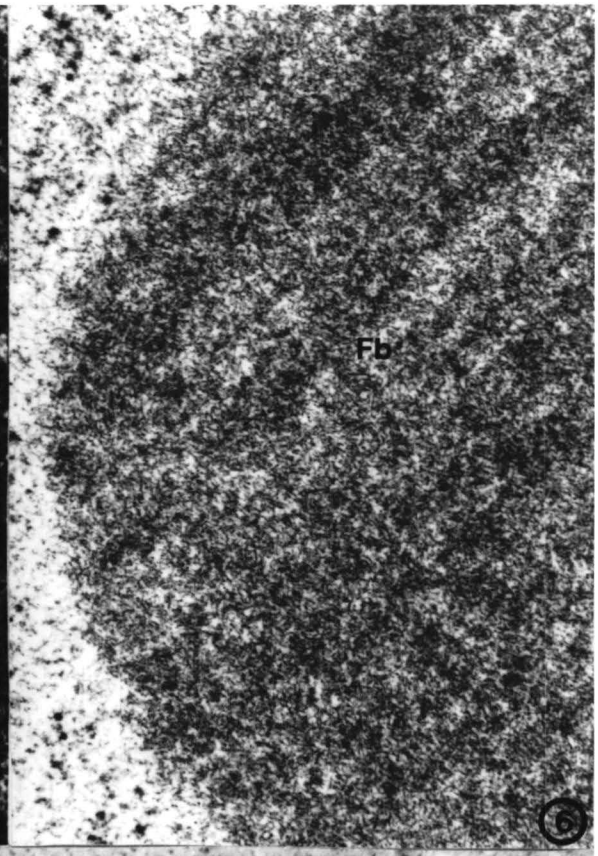
Fig. 5. Previtellogenic (PV) oocyte. Note density of fibrillar component fibers. Gc= granular component of nucleolus, Fc= fibrillar component of nucleolus. x57,000.

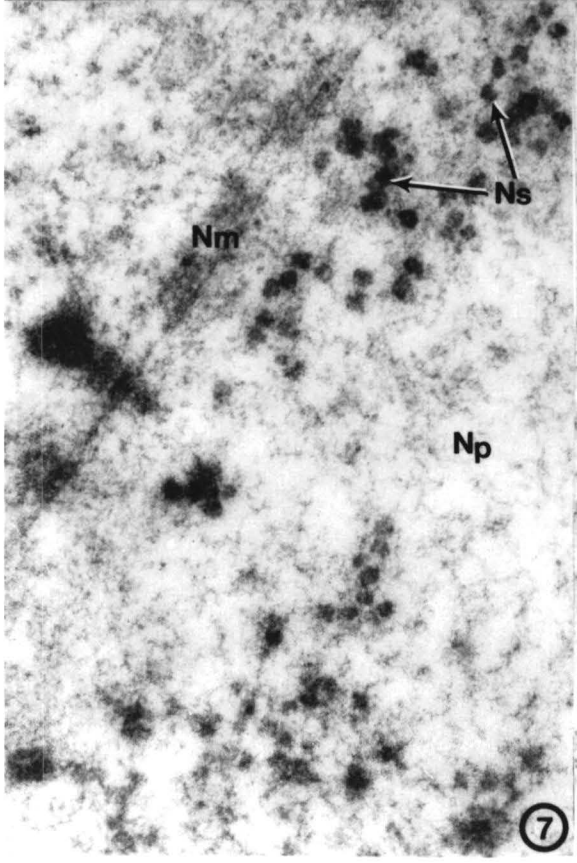
Fig. 6. Previtellogenic (PV) oocyte nucleus. Fb= fibrillar body. x21,300.

Fig. 7. Early vitellogenic (EV) oocyte. Note density of nucloplasmic fibers. Np= nucleoplasm, Ns= nucleosomes, Nm= nuclear membrane. x57,000.

Fig. 8. Early vitellogenic (EV) oocyte nucleus. Note number of nucleosomes. x7,500.


Gc
Fc
5


Fb
6


Ns
Nm
Np
7

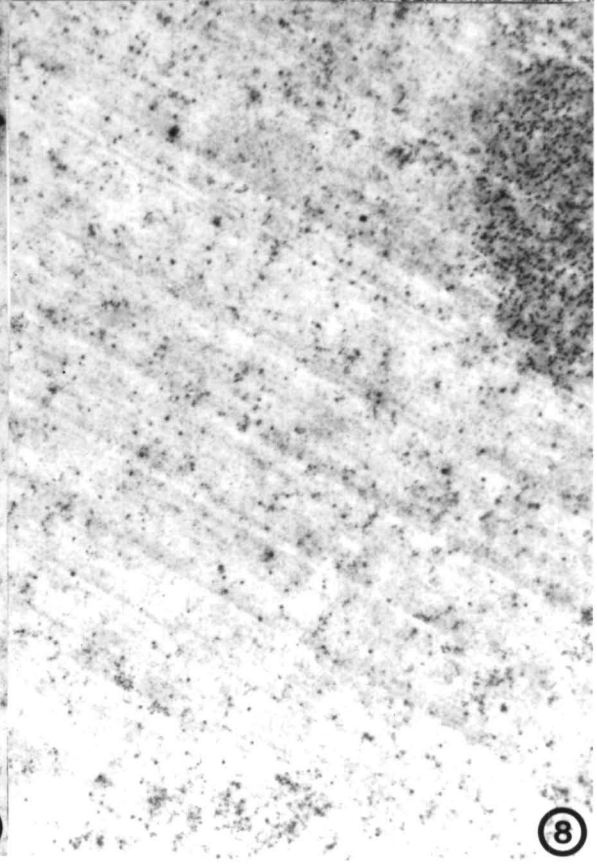

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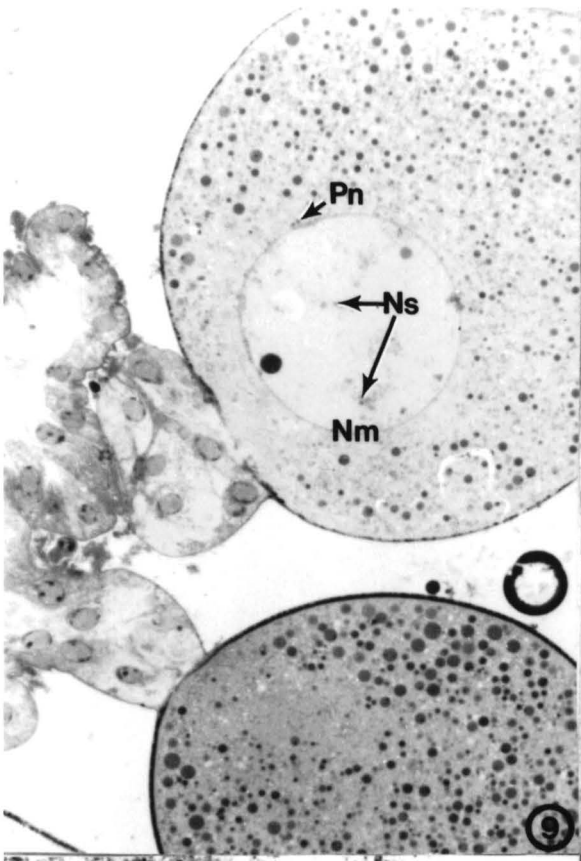
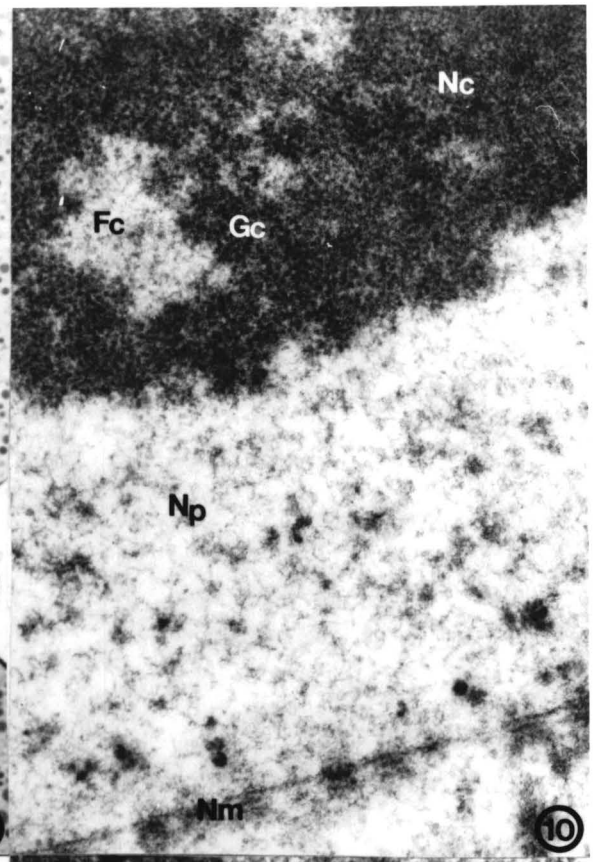
Fig. 9. Early vitellogenic (EV) oocyte, light photomicrograph. Note clusters of nucleosomes (Ns) and perinuclear bodies associated with cytoplasmic side of nuclear membrane. Pn= perinuclear bodies, Nm= nuclear membrane. x160.

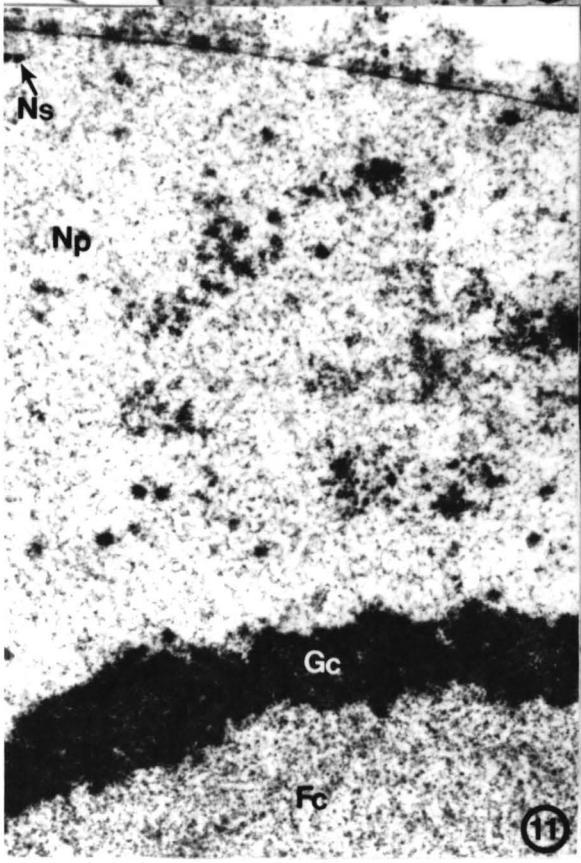
Fig. 10. Early vitellogenic (EV) oocyte. Note fiber density in vacuolar region of nucleolus in comparison with nucleoplasmic fibers. Nc= nucleolus, Nm= nuclear membrane, Fc= fibrillar component of nucleolus, Gc= granular component of nucleolus, Np= nucleoplasm. x45,000.

Fig. 11. Late vitellogenic (LV) oocyte nucleus, fed, unmated (FU). Note density of fibers and lack of nucleosomes in nucleoplasm and density of fibers in fibrillar component of nucleolus. Fc= fibrillar component of nucleolus, Gc= granular component of nucleolus, Np= nucleoplasm, Ns= nucleosome. x27,300.

Fig. 12. Late vitellogenic oocyte, fed, mated (FM). Perinuclear bodies are seen outside porous nuclear membrane. Pn= perinuclear body, Nm= nuclear membrane, Cp= cytoplasm. x45,000.


Pn
Ns
Nm
9


Nc
Fc
Gc
Np
Nm
10


Ns
Np
Gc
Fc
11

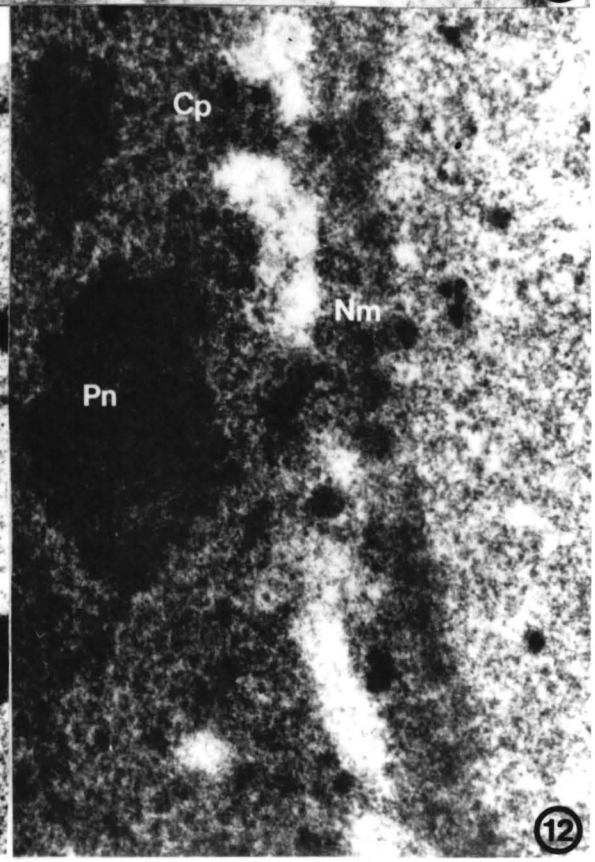
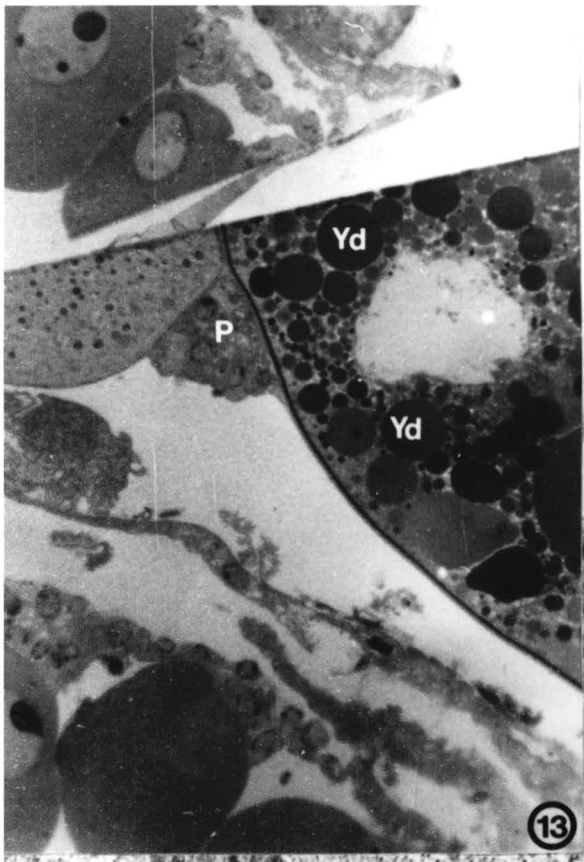

Cp
Nm
Pn
12

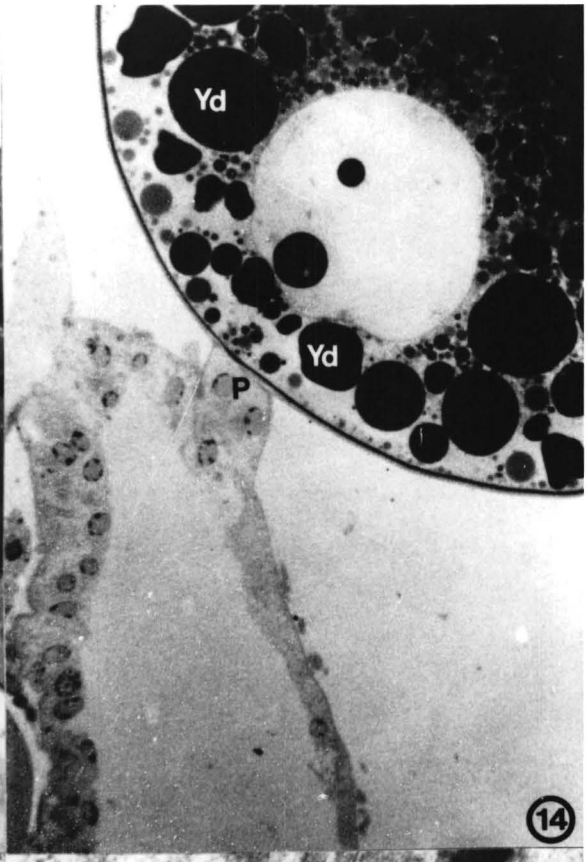
Fig. 13. Late vitellogenic (LV) oocyte, fed, mated (FM); light photomicrograph. Nucleus shows light staining characteristic of this stage. Yolk droplets are seen close to oocyte periphery at pedicel-oocyte interface. Fewer clusters of granules are seen in comparison with early vitellogenic stage. Yd= yolk droplets, P= pedicel. x160.

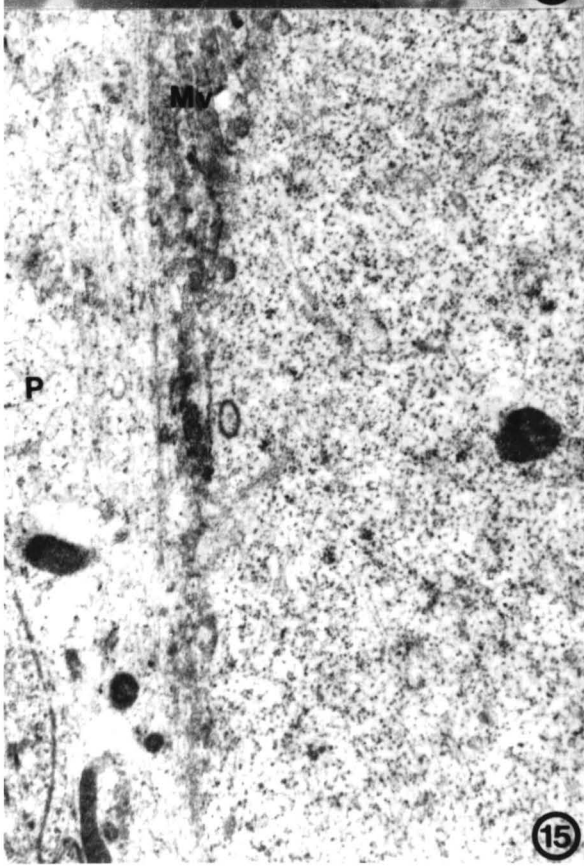
Fig. 14. Pedicel - late vitellogenic (LV) oocyte interface; fed, unmated (FU) female. Altered staining ability of nucleus evident. Two nucleoli present. No yolk droplets near attachment point. Yd= yolk droplets, P= pedicel. x160.

Fig. 15. Pedicel - previtellogenic (PV) oocyte interface. Note evenly distributed ribosomes in oocyte and microvilli from oocyte. P= pedicel, Mv= microvilli. x17,100.

Fig. 16. Pedicel - early vitellogenic (EV) oocyte interface. Polyribosomes are seen in oocyte cytoplasm and microvilli extend from both pedicel cell and oocyte membranes. P= pedicel, Mv= microvilli, Pr= polyribosomes. x45,000.


Yd
P
Yd
13


Yd
Yd
P
14


Mv
P
15

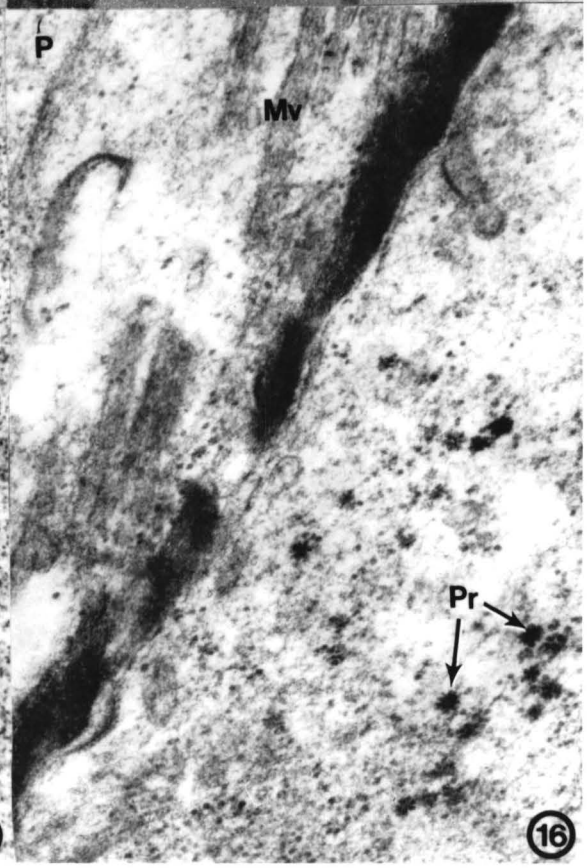
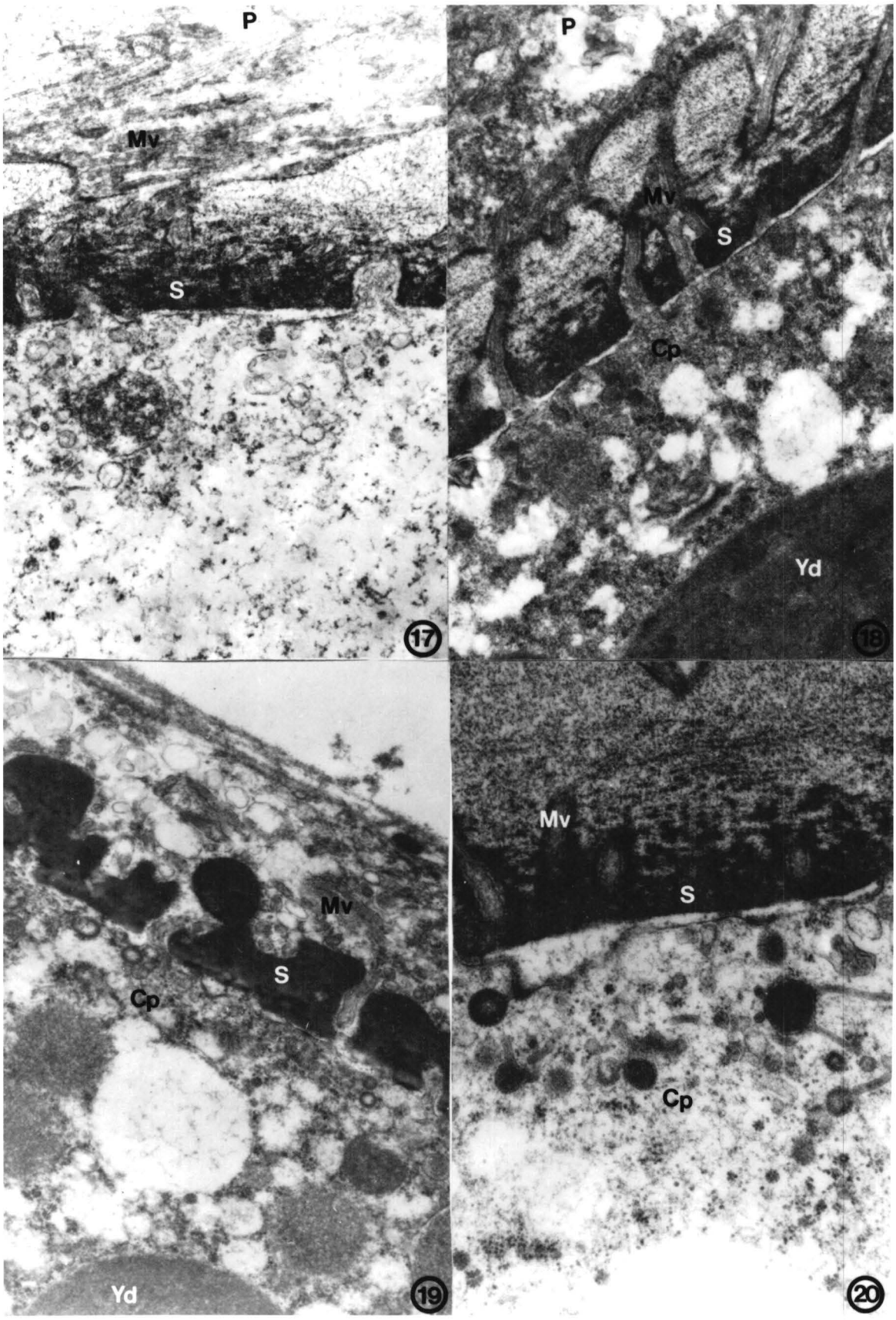

P
Mv
Pr
16

Fig. 17. Pedicel - late vitellogenic (LV) oocyte interface; fed, unmated (FU) female. P= pedicel, Mv= microvilli, S= shell. x27,300.

Fig. 18. Pedicel - late vitellogenic (LV) oocyte interface; fed, mated (FM) female. Note dense oocyte cytoplasm displaced by developing yolk droplets. Cp= cytoplasm, P= pedicel, Mv= microvilli, S= shell, Yd= yolk droplets. x22,000.

Fig. 19. Late vitellogenic (LV) oocyte at other than pedicellary area; fed, mated (FM) female. Note dense cytoplasm displaced by developing yolk droplets. Cp= cytoplasm, Mv= microvilli, S= shell, Yd= yolk droplets. x22,000.

Fig. 20. Late vitellogenic (LV) oocyte, fed, mated (FM) female. Note lack of dense cytoplasm due to dearth of yolk droplets. Cp= cytoplasm, Mv= microvilli, S= shell. x30,000.


P
Mv
S
17
P
Mv
S
Cp
Yd
18
Mv
S
Cp
Yd
19
Mv
S
Cp
20