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Ornithodoros moubata: Spermateleosis and Secretory Activity of the Sperm

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SAHLI, R., GERMOND, J. E., AND DIEHL, P. A. 1985. *Ornithodoros moubata:* Spermateleosis and secretory activity of the sperm. *Experimental Parasitology* **60**, 383–395. Cytological aspects of spermateleosis in the tick *Ornithodoros moubata* were studied by electron microscopy. During spermateleosis, detachment of the operculum from the outer sheath of the prospermium results from the fusion of the plasma and the cisternal membranes. The fusion occurs between the shoulder of the acrosomal vesicle and the electron-dense layer of the operculum. A factor inducing vitellogenesis and egg-laying is secreted by the sperm cell after spermateleosis, and begins after the cell is almost completely devaginated. *In vitro,* fully devaginated spermiophores secrete most of this factor during the first 12 hr of incubation. The vitellogenesis-inducing activity of the secretion is sensitive to proteinase K (EC 3.4.21.14) digestion and correlates with the presence of two high-molecular-weight proteins in the sperm cell incubation medium.

INDEX DESCRIPTORS AND ABBREVIATIONS: *Ornithodoros moubata;* Tick; Acarina; Ixodoidea; Argasidae; Sperm cell, prospermium, spermiophore; Sperm maturation; Spermateleosis; Vitellogenesis inducing factor (VIF).

INTRODUCTION

The study of reproduction in ticks has led to the observation of unusual phenomena such as the complicated morphogenesis of the sperm cell (for review see Oliver and Shepherd 1981; Oliver 1982) and its influence in triggering egg-laying in *Ornithodoros moubata* (Aeschlimann 1968; Germond and Aeschlimann 1977).

At the final stage of differentiation in the male, the sperm (prospermium) is a rod-shaped cell with an outer sheath containing a large cisternal cavity which surrounds an invaginated inner core. The posterior part of the prospermium is closed and has a truncated end. The tip of the anterior part has a "cap-like structure" (Oliver and Brinton 1972) or operculum (Borut and Feldman-Muhsam 1976) which is separated from the outer sheath by a rim of one or a few ridges. During mating, the prospermia come into contact with the secretions of the male accessory gland complex in the endospermatophore. One of these secretions (Shepherd *et al.* 1982) induces the first step of the post-ejaculatory spermiogenesis (Pinkerton *et al.* 1982), i.e., the opening of the operculum, or spermateleosis (Borut and Feldman-Muhsam 1976). This aperture allows the inner core to slide out and thus to double the length of the cell. Then, the sperm becomes mobile (turning into a spermiophore) and completes its differentiation in the female genital tract.

In *O. moubata,* Aeschlimann (1968) found that injection of sperm cells (either intact or as homogenates) into the body cavity of virgin females that had been fed previously induced vitellogenesis and egg-laying. Germond and Aeschlimann (1977) showed that this reaction is due to a heat-labile factor of high molecular weight re-

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leased by the sperm cells. In this report, we call this factor VIF.

This paper describes the ultrastructural aspects of events leading to the opening of the operculum of the prospermium. We have also shown that the sperm cells secrete the VIF only after this event, and that the presence of VIF correlates strongly with the appearance of specific high-molecular-weight proteins in a sperm cell culture medium.

Materials and Methods

Ornithodoros moubata (Murray 1877; Walton 1962) were reared as described by Geigy and Herbig (1955) and by Germond and Aeschlimann (1977).

For transmission electron microscopy, female *O. moubata* were dissected in cold TC 199 (Hank's without L-glutamine: Seromed) immediately after copulation. Intact endospermatophores were removed and fixed at room temperature in cacodylate buffer (0.1 *M* cacodylate pH 7.2, 4 m*M* $MgCl_2$, 4 m*M* $CaCl_2$) containing 4% sucrose and 2.5% glutaraldehyde. After fixation, the endospermatophores were washed in five changes of 0.2 *M* cacodylate buffer containing 4% sucrose, the first four changes for 5 min at room temperature, the last wash at 4 C overnight. They were then postfixed with 1% osmium tetroxide in 0.1 *M* cacodylate buffer containing 4% sucrose, washed, dehydrated, and embedded in epoxy resin (Spurr, Taab Laboratories). Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 201 transmission electron microscope equipped with a goniometer stage.

For scanning electron microscopy, prospermia were taken from the vesicula seminalis of fed males. The sperm cells were washed in cold TC 199, fixed, and postfixed as described above. After dehydration with acetone, the sperm cell suspension was critical point dried. After coating with carbon and gold, the sperm cells were examined in a Philips 500 scanning electron microscope.

For the incubation experiments, endospermatophores were dissected out of impregnated females. The sperm cells were released and washed three times in TC 199, recovered by centrifugation at 50 *g* for 30 sec, and then resuspended in 10 µl of TCPa (1 vol of 0.2 *M* NaK phosphate buffer, pH 7.5, 1 vol of TC 199, 6 µg/ml streptomycin, 18 µg/ml penicillin) per endospermatophore equivalent. One endospermatophore equivalent corresponds to the number of sperm cells contained in one endospermatophore. At that cell concentration, the VIF was arbitrarily considered as being at a 1/1 relative concentration. The suspension was kept in sterile 1.5 ml Eppendorf tubes at 28 C, and less than 500 µl was added per tube. The viability of the cells was determined using trypan blue, and in our conditions, they remained viable for at least 2 days. The devagination stage of the sperm cells was estimated from the percentage of the length along which the inner core had evaginated.

After incubation, the sperm cells were removed by low speed centrifugation, and the supernatant cleared from any cells or debris by centrifugation at 10,000*g* for 10 min. The supernatant was kept at 4 C until injection into the body cavity of fed virgin females (bioassay) as described by Germond and Aeschlimann (1977). The inoculum consisted of 2 µl of VIF-containing medium or medium alone in control experiments. Dilutions were made in TCPa or TCPb (TCPa supplemented with 100 µg/ml bovine serum albumin) unless otherwise specified. The injected females were kept isolated at 28 C in the dark for 10 days, and a response was judged positive by the appearance of either egg-laying, or Stages 4 or 5 of vitellogenesis (Balashov 1972). At Stage 4, the eggs have incorporated vitellus and are ready to be ovulated. At Stage 5, the eggs are in the lumen of the ovary or the oviduct or in the uterus, and are ready to be laid.

Slab gel electrophoresis in 12% acrylamide was performed as described by Laemmli and Favre (1973). The proteins in culture supernatants were precipitated in 5% trichloroacetic acid for 30 min at 4 C. They were recovered by centrifugation at 10,000*g* for 10 min and washed successively with ethanol, ethanol–ether (1:1), and ether. After drying, the protein pellet was resuspended in sample buffer (0.1 *M* Tris–HCl, pH 6.8, 2% SDS, 2 m*M* DTT, 10% glycerol, and 0.001% bromophenol blue) and placed in a boiling water bath for 5 min before loading. The gel was stained with Coomassie blue R 250.

Digestion of sperm supernatants was carried out using proteinase K (EC.3.4.21.14) purchased from Boehringer Mannheim GmbH. Sperm cells were obtained from females left with males for 48 hr. The cells were resuspended in 30 m*M* Tris–HCl pH 7.6, 200 m*M* NaCl at a density yielding VIF at a 2/1 relative concentration and incubated for 3 hr at room temperature. The cells were judged to be alive after that incubation by trypan blue exclusion. After centrifugation of the cells, 100 µl of supernatant was digested with 8 µg of proteinase K in a 200 µl reaction mixture (the VIF was then at a 1/1 relative concentration) containing 30 m*M* Tris–HCl, pH 7.6, 200 m*M* NaCl, and 2 m*M* EDTA at 30 C for 1 to 6 hr. After incubation, 20-µl aliquots were taken for injection (bioassay). The dilutions were made in digestion buffer without EDTA.

Results

The early steps of sperm maturation in the female *Ornithodoros moubata* genital tract are schematically represented in Fig.

1. The prospermium contains a large cisternal cavity which divides the cell into an outer sheath and an invaginated inner core. Many cellular processes project into the cavity (Figs. 1a, 2c, 8a) which is filled with a filamentous structure (Fig. 4) and an ordered structure just under the operculum (Fig. 2c). After ejaculation, the opening of the operculum (Fig. 1b) is followed by the evagination of the inner core (Figs. 1c, d). At the same time, formation of the acrosomal canal is achieved by the invagination of a part of the outer sheath into the inner core.

In the anterior part of the prospermium, a furrow indicates the junction between the outer sheath and the operculum (Figs. 2b, c, 3, 8a). Seen at a higher magnification (Fig. 3), the inner wall of the operculum is delimited by the cisternal membrane, tightly bordered in the cytoplasm by a thick electron-dense layer which ends at the level of the furrow. The outer wall of the operculum is delimited by the plasma membrane, placed at a constant distance from the electron-dense layer. At the level of the furrow, two thinner unit membranes fold upon themselves and enclose the acrosomal vesicle. This structure is summarized in Figs. 8a, b.

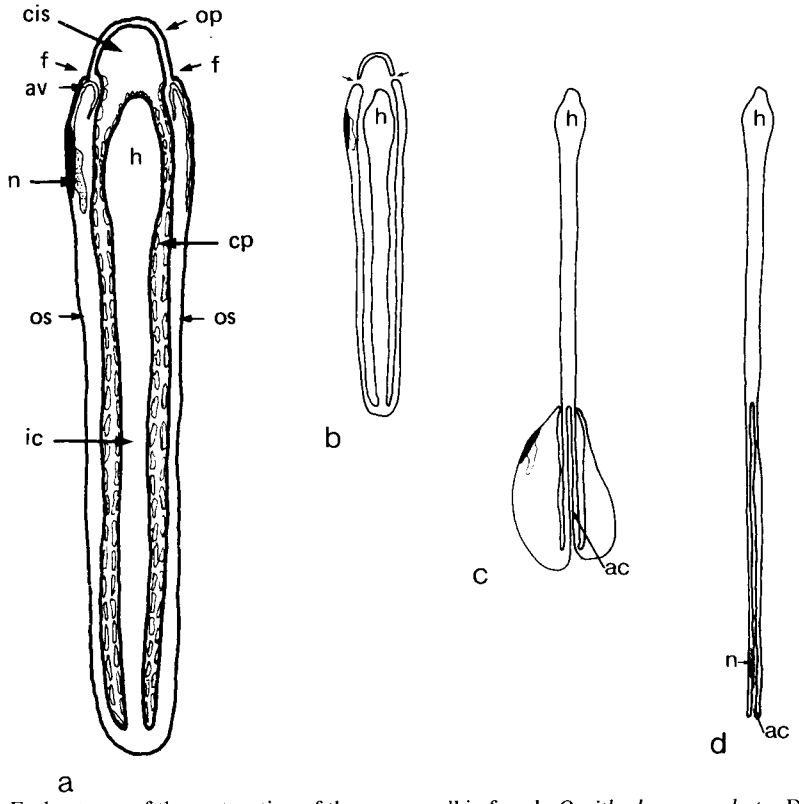


FIG. 1. Early stages of the maturation of the sperm cell in female *Ornithodoros moubata*. Drawings are not to scale. (a) Prospermium before spermateleosis. (b) Prospermium after spermateleosis, the operculum is detached from the outer sheath (arrows). (c) Devagination of the inner core through the aperture of the outer sheath and simultaneous formation of the acrosomal canal by invagination of the outer sheath into the inner core. (d) Mobile spermiophore in the female genital tracts. The acrosomal vesicle and the cellular processes are not drawn in (b, c, and d). ac, acrosomal canal; av, acrosomal vesicle; cis, cisterna; cp, cellular processes; f, furrow; h, head of the spermiophore; ic, inner core; n, nucleus; op, operculum; os, outer sheath.

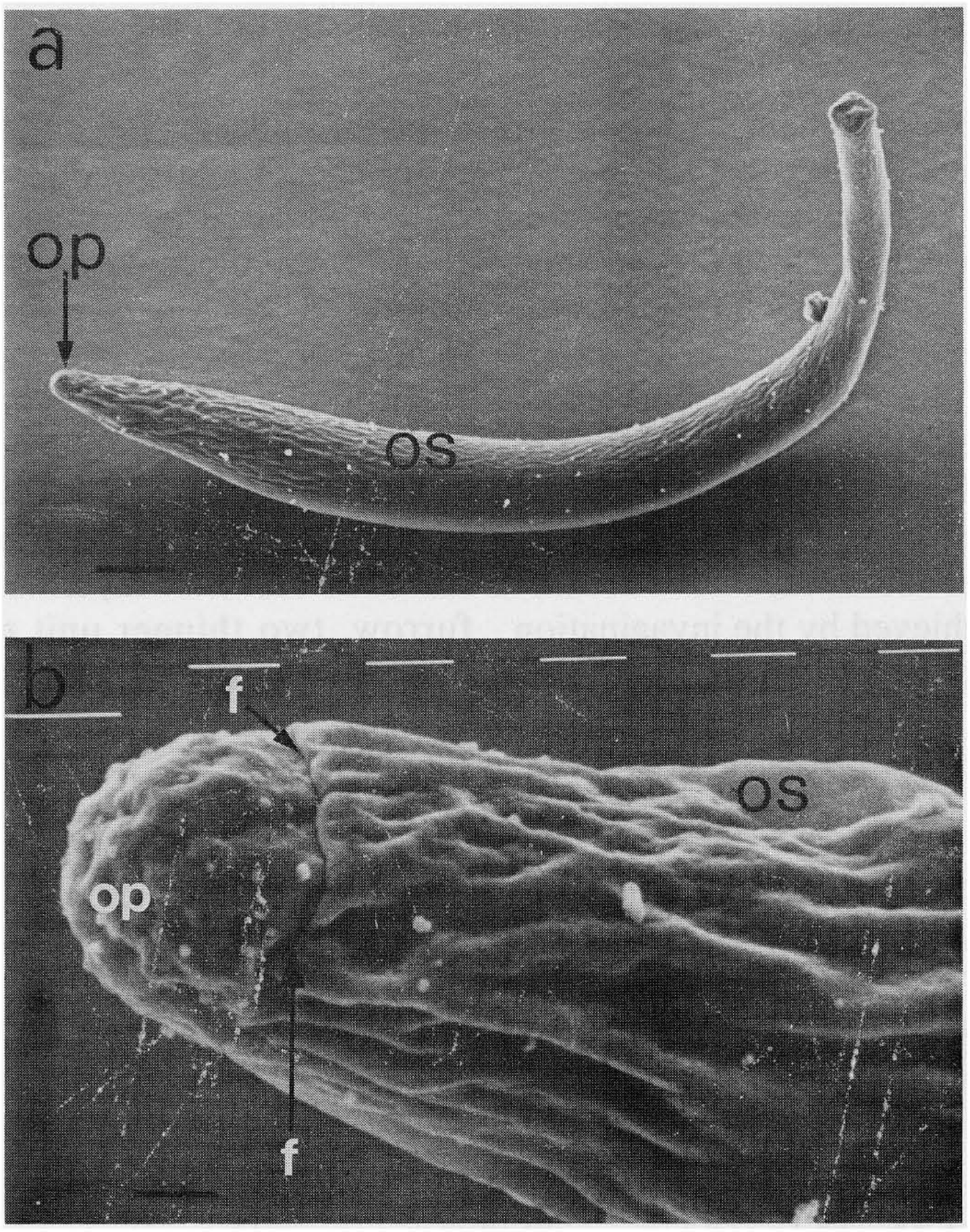
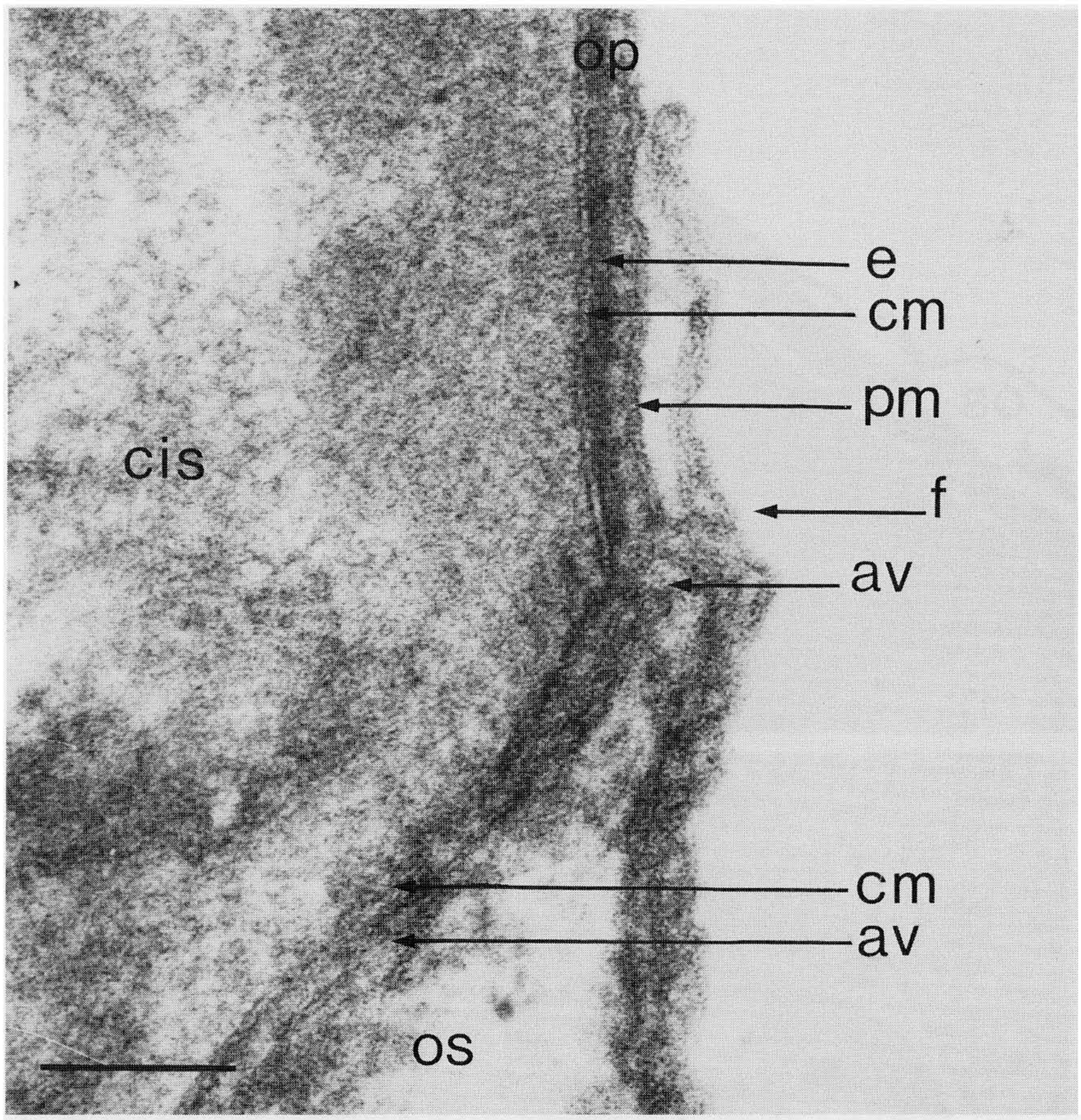


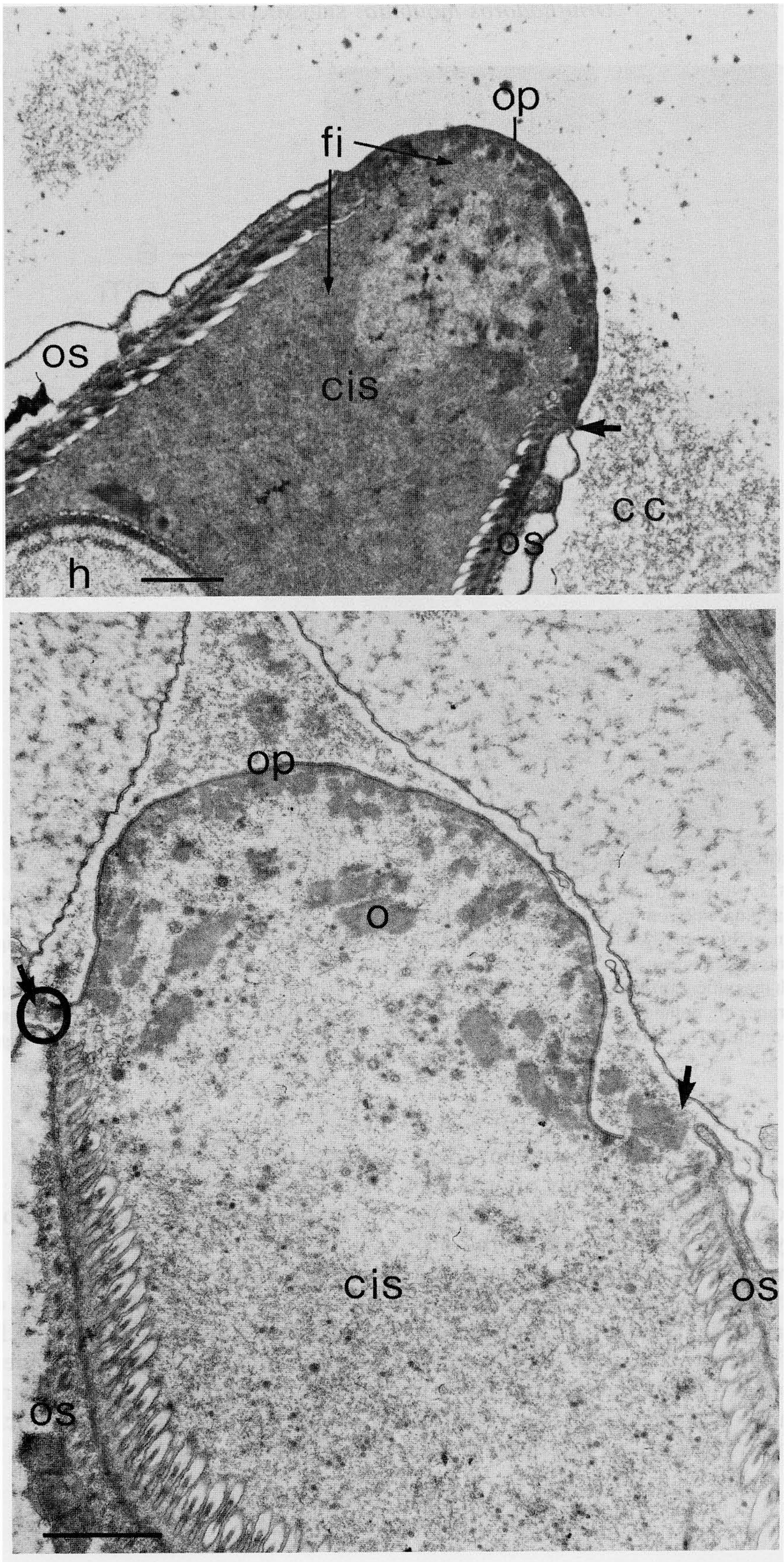
FIGURE 2.



FIGS. 2 AND 3. Ultrastructure of the anterior part of the prospermium of *Ornithodoros moubata*. (Fig. 2a) Scanning electron micrograph of a prospermium, the operculum is on the left. (Fig. 2b) Magnification of Fig. 2a showing the operculum separated from the outer sheath by a furrow. (Fig. 2c) Transmission electron micrograph of a longitudinal section through the area shown in Fig. 2b. (Fig. 3) High magnification of the area encircled in Fig. 2c. av, acrosomal vesicle; cis, cisterna; cm, cisternal membrane; cp, cellular processes; e, electron-dense layer; f, furrow; o, ordered structural elements; op, operculum; os, outer sheath; pm, plasma membrane. Bar = 10 μm (Fig. 2a) or 1 μm (Figs. 2b, c, and 3).

During mating, the prospermium opens at the level of the furrow only after it had been in contact with secretions of the male accessory gland complex. Then, the cisternal content is expelled from the cell, and the operculum becomes detached from the outer sheath (Fig. 4). The cisternal content loses its filamentous structure and some of the ordered structural elements do not remain attached to the operculum (Fig. 5). This opening starts at one side (Fig. 4) and extends to the other side of the prospermium (Fig. 5).

At the beginning of spermateleosis (Fig. 6), the end of the electron-dense layer is rather distant from the acrosomal vesicle. At this level, the plasma and the cisternal membranes are very close together suggesting a membrane fusion. Indeed, at a high magnification (Fig. 7), the extremity of the detached operculum (area encircled in Fig. 5) shows that the cisternal and the



FIGURES 4 AND 5.

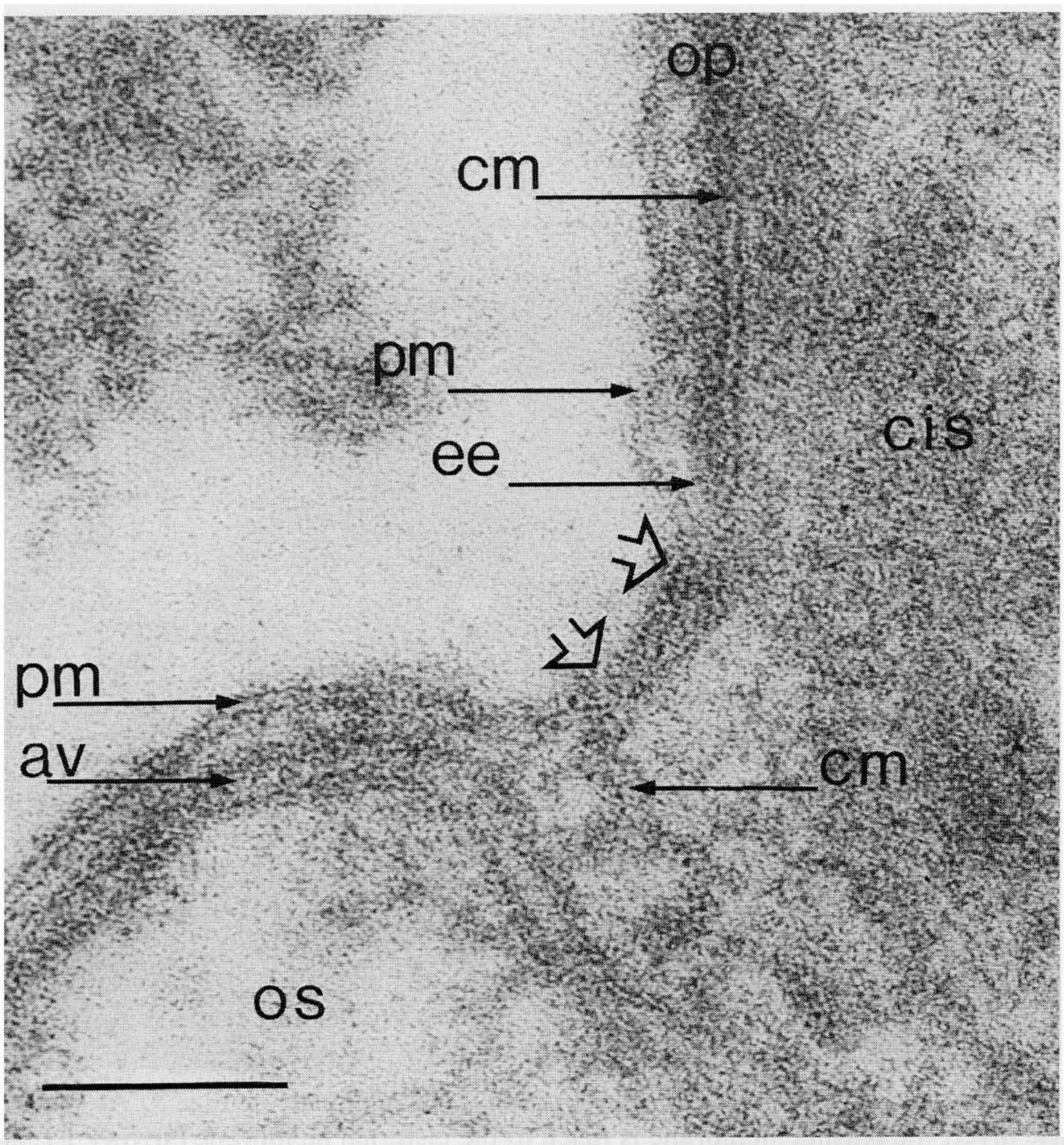


FIG. 6. High magnification of the junction between the operculum and the outer sheath of a prospermium of *Ornithodoros moubata,* presumably at the beginning of spermateleosis. The plasma and the cisternal membranes are very close together at the end of the electron-dense layer (arrows) suggesting a membrane fusion. av, acrosomal vesicle; cis, cisterna; cm, cisternal membrane; ee, end of the electron-dense layer; op, operculum; os, outer sheath; pm, plasma membrane. Bar = 0.1 μm.

plasma membranes are linked together confirming a fusion of these membranes. This extremity corresponds exactly to the termination of the electron-dense layer. Figures 8c–d show a schematic view of the detachment of the operculum.

The production of VIF was followed during the first hours after mating. In this experiment, females were isolated immediately after mating and kept for a period varying from 1 to 48 hr. The endospermatophores were then recovered by dissection, the sperm cells were washed and incubated for 12 hr *in vitro,* and their devagination stage was observed before and after incubation. At the end of the incubation period, the sperm cells were centrifuged, and the VIF was detected in the supernatants by the bioassay (see Materials and Methods). Three dilutions of the superna-

FIGS. 4 AND 5. Detachment of the operculum from the outer sheath of the sperm of *Ornithodoros moubata*. The detachment of the operculum starts at one side of the prospermium (arrow, Fig. 4) proceeds along the furrow and reaches the other side of the cell (arrows, Fig. 5). The area encircled in Fig. 5 is shown at a higher magnification in Fig. 7. cc, cisternal content; cis, cisterna; fi, filamentous structure; h, head of the spermiophore; o, ordered structural elements; op, operculum; os, outer sheath. Bar = 1 μm (Fig. 4) or 0.1 μm (Fig. 5).

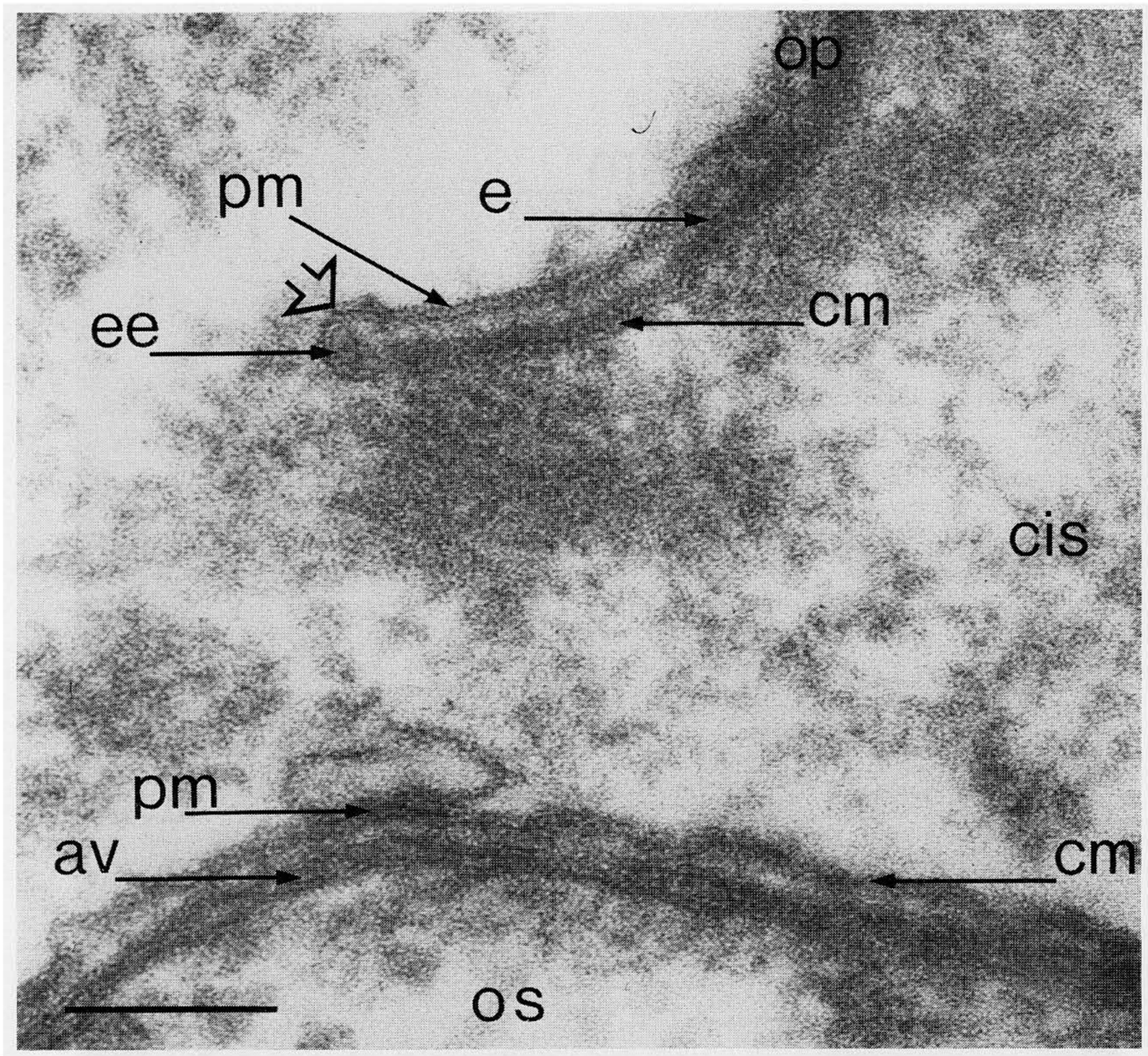


FIG. 7. Ultrastructure of the extremity of the operculum encircled in Fig. 5. At the end of the electron-dense layer, the plasma membrane is fused to the cisternal membrane (arrow). av, acrosomal vesicle; cis, cisterna; cm, cisternal membrane; e, electron-dense layer; ee, end of the electron-dense layer; op, operculum; os, outer sheath; pm, plasma membrane. Bar = 0.1 μm.

tants were assayed (1/1, 1/10, and 1/100) using two fed virgin females per dilution. There was no detectable VIF in the incubation medium of sperm cells taken 1 hr after copulation, as none of the injected dilutions induced a positive response in the bioassay (Table I). At the beginning of the incubation, the cells had started to devaginate (on average, 10% of the length of the sperm cell was devaginated) and at the end they were almost completely devaginated (80% of the length). Since the cisternal contents were released into the culture medium during this period, it is apparent that the VIF could not be contained in them. In contrast, the VIF was detected at least at the lowest dilution in another series of supernatants tested in the bioassay. These supernatants originated from the incubation *in vitro* of sperm taken from females impregnated from 12 to 48 hr prior to dissection. These cells were almost completely devaginated (80 to 100% of the length) and free of any cisternal content at the beginning of incubation. Therefore, the VIF was specifically secreted by the sperm cell after its devagination was almost if not completely finished.

In another experiment, the appearance of the VIF was correlated with the protein composition of the incubation medium containing sperm cells. The cells were obtained from females left with males for 6 hr and kept isolated the following 17 hr. Sperm cells from 150 endospermatophores were resuspended in 1 ml of TCPa and immedi-

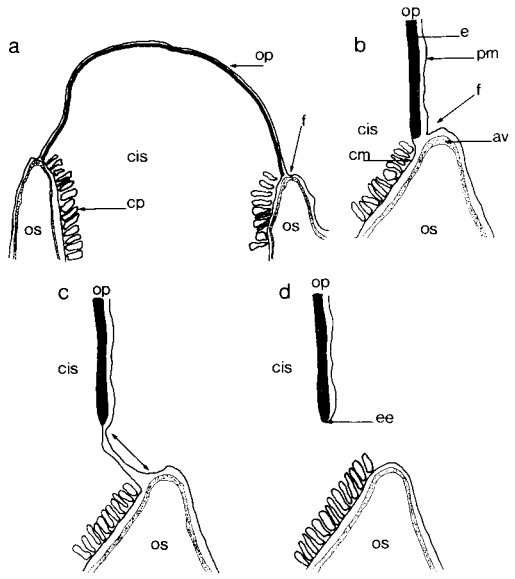


FIG. 8. Schematic representation of spermateleosis in *Ornithodoros moubata*. (a) General organization of the anterior part of the prospermium. (b) The junction of the operculum with the outer sheath is shown at a higher magnification. In the operculum, the cisternal membrane is tightly bordered by an electron-dense layer which ends where the acrosomal vesicle folds upon itself in the outer sheath. The distance between the cisternal and the plasma membranes remains constant in the operculum whereas it varies in the outer sheath. (c) The distance between the end of the electron-dense layer and the shoulder of the acrosomal vesicle increases prior to the fusion of the cisternal and the plasma membranes leading to the detachment of the operculum from the outer sheath (d). av, acrosomal vesicle; cis, cisterna; cm, cisternal membrane; cp, cellular processes; e, electron-dense layer; f, furrow; op, operculum; os, outer sheath; pm, plasma membrane.

ately centrifuged at low speed. The supernatant (S1) was tested for the presence of the VIF by the bioassay, and its protein composition analyzed by electrophoresis in a 12% slab gel under denaturing conditions (Fig. 9). The cells were resuspended in the same volume of fresh TCPa and incubated *in vitro*. Every 12 hr, this change of medium was repeated until four consecutive supernatants were obtained (S2–S5). For the bioassay, three dilutions of each supernatant were made in TCPb (1/5, 1/25, and 1/125), and three fed virgin females used per dilution. Positive responses were found for S1, S2, and S3 (Table II), showing that the VIF is secreted in the first 24 hr of incubation *in vitro* of fully devaginated spermiophores. S2 contained the highest concentration of VIF since it was still positive at the highest dilution tested in the bioassay, whereas in S1 and S3, the vitellogenesis inducing (VI) activity was lost upon dilution. If the quantity of VIF is estimated as the inverse function of the highest positive dilution, decreasing quantities of VIF would be found in S2, S1, and S3, respectively. This result shows that the VIF is secreted by the devaginated sperm cells mostly during the first 12 hr of their incubation. A gel electrophoretic analysis of the proteins found in the same supernatants is shown in Fig. 9. S1 contained many proteins derived from the content of the endospermatophores (secretions from the male accessory glands and content of the cisterna of the prospermium). Therefore, the content of only 30 endospermatophores was used for the protein analysis. In S2–S5, the proteins released from the sperm cells of 145 endospermatophores were analyzed. Two proteins of high molecular weight (above 100K; P1 and P2) clearly distinguished S2 from the other supernatants. Taking into account the lower volume of supernatant analyzed from S1 and using the protein shown by an asterisk as a standard, P1 and P2 would be found in decreasing quantity in the order S2, S1, S3, S4, and S5, in correlation with the estimation of the quantity of the VIF in these supernatants.

To check the correlation between proteins and VIF, we digested a sperm cell culture supernatant with proteinase K (see Materials and Methods). Four dilutions of each reaction medium (1/1, 1/4, 1/16, and 1/64) were tested in the bioassay, and three fed virgin females used per dilution. The VIF incubated for 6 hr in absence of proteinase K was fully active up to a 1/16 dilution (Table III). However, the VI activity

TABLE I
Secretion of the Vitellogenesis Inducing Factor *in Vitro* by Sperm Cells Taken from Female *Ornithodoros moubata* 1 to 48 hr after Mating

Time of recovery of the sperm cell after mating (hr)	Davagination stage of the sperm cell before and after incubation		Dilutions tested	Response in bioassay[a]
Control				−, −
			1/1	−, −
1	10%	80%	1/10	−, −
			1/100	−, −
			1/1	+, +
12	80%	100%	1/10	+, −
			1/100	+, −
			1/1	+, +
24	100%	100%	1/10	+, −
			1/100	+, −
			1/1	+, +
36	100%	100%	1/10	+, −
			1/100	+, −
			1/1	+, +
48	100%	100%	1/10	+, +
			1/100	−, −

[a] Two females per dilution were injected with the indicated dilutions of the incubation media. Positives and negatives were scored as described under Materials and Methods.

was gradually lost during the 1, 3, and 6 hr of proteinase K digestion. As a control, injection of 1/1 proteinase K-treated supernatant did not abolish egg-laying of freshly impregnated females (data not shown). In a parallel experiment, proteinase K was shown to be active with bovine serum albumin as a substrate, the plateau being reached after 6 hr (data not shown).

These observations suggest that the VI activity may be correlated with the presence in the sperm cell incubation medium of at least the two high-molecular-weight proteins P1 and P2 secreted by the sperm cell.

Discussion

Scanning electron micrographs (Pinkerton *et al.* 1982) show that the external structure of the prospermium of *Ornithodoros moubata* is very similar to that of an Ixodid *Amblyomma hebraeum* (Wüest *et al.* 1978). The prospermium can be divided into two parts. The anterior part, or operculum, is very short compared to the posterior, or outer sheath, and separated from it by a furrow. Removal of the operculum during spermateleosis (Borut and Feldman-Muhsam 1976) allows the inner core to evaginate through the aperture of the outer sheath and hence the maturation of the sperm cell from prospermium to spermiophore. Shepherd *et al.* (1982) showed that this step was triggered by a 12.5 kDa polypeptide from the male accessory glands and suggested that a membrane fusion may be the mechanism by which the operculum is removed. We have confirmed that the removal of the operculum is due to the fusion of the plasma membrane and the cisternal membrane. This fusion takes place between the electron-dense layer of the operculum and the shoulder of the acrosomal vesicle in the outer sheath. This process thus closely resembles exocytosis.

The electron-dense layer has been described as a unit membrane fused to the cisternal membrane in *O. gurneyi* (Feld-

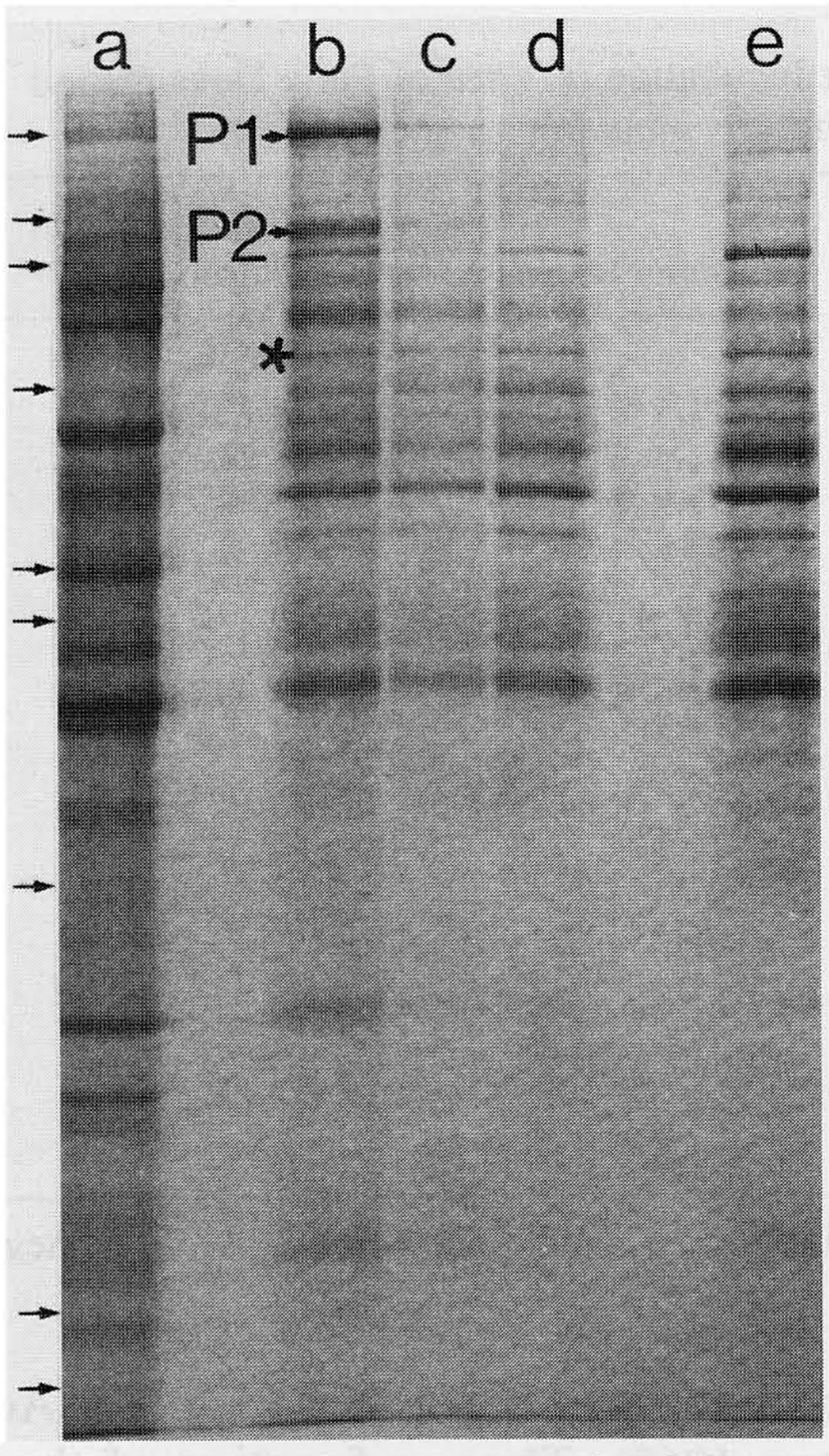


FIG. 9. Gel electrophoretic analysis of the proteins found in incubation media of fully devaginated spermiophores from *Ornithodoros moubata*. Lanes a–e, respectively, S1–S5. The proteins labeled P1 and P2 are enriched in S2. This enrichment correlates with the strong vitellogenesis-inducing activity exhibited by S2 (Table II). The protein shown by an asterisk was used as a standard to estimate the relative proportions of P1 and P2 in the different incubation media. The molecular weight markers (arrows) are in descending order: myosin (200K), β-galactosidase (117K), phosphorylase B (94K), bovine serum albumin (66K), chicken albumin (45K), ovalbumin (43K), chymotrypsinogen (25K), horse myoglobin (18K), and cytochrome *c* (12K).

man-Muhsam and Filshie 1979). Through our observations on *O. moubata,* we propose another interpretation of the ultrastructure of the operculum. We believe that the cisternal-oriented part of the operculum is made of the cisternal membrane to which a thick structural layer (the electron-dense layer) is juxtaposed in the cytoplasm. This layer is clearly thicker and does not elicit the usual structure of a unit membrane. It could serve as a guide during the fusion of the membranes leading to the separation of the operculum from the outer sheath. As in *O. gurneyi,* we have observed filaments in the cisterna and an ordered structure, described as paracrystals by the same authors, just under the operculum.

During maturation of the sperm cell, we have observed that as soon as the cell is almost completely devaginated it secretes the VIF during 12 to 24 hr of incubation. The presence of the two high-molecular-weight proteins (P1, P2) in bioassay positive supernatants and the sensitivity of the VI activity to proteinase K digestion are consistent with the heat sensitivity of the VI activity observed by Germond and Aeschlimann (1977). The correlation between the VI activity and these proteins does not exclude, however, the possibility of the interaction of another factor. This factor could be inactive if it is not bound to an intact protein carrier.

We have noticed that the sperm cells begin to secrete the VIF once they are almost fully devaginated. The majority of the P1 and P2 proteins are also secreted once the sperm cell has eliminated the cisternal content. These observations suggest that the spermiophores secrete the VIF outside of the endospermatophore when they migrate in the female genital tract. Germond and Aeschlimann (1977) found that injection of seminal vesicle homogenates in fed virgin females led to strong vitellogenesis and often to egg-laying. The VIF is thus synthesized in the male before copulation and is then specifically secreted by the spermiophores in the female to induce full vitellogenesis and egg-laying.

In contrast to most Ixodids, copulation is not a prerequisite for successful feeding in Argasids (for review see Diehl *et al.* 1982). Argasid-fed virgin females begin vitellogenesis after their blood meal. If copulation does not occur within the next month, the females will resorb their eggs leading to abortive vitellogenesis. However, copulation or injection in their hemocoel of the

TABLE II
Detection of the Vitellogenesis Inducing Factor during Incubation *in Vitro* of Fully Devaginated Spermiophores from *Ornithodoros moubata*

Supernatant tested	Period of incubation (hr)	Dilutions tested	Response in bioassay[a]
Control			−,−,−
		1/5	+,+,+
S1	—	1/25	+,+,−
		1/125	−,−,−
		1/5	+,+,+
S2	0–12	1/25	+,+,−
		1/125	+,+,−
		1/5	+,+,+
S3	12–24	1/25	−,−,−
		1/125	−,−,−
		1/5	−,−,−
S4	24–36	1/25	−,−,−
		1/125	−,−,−
		1/5	−,−,−
S5	36–48	1/25	−,−,−
		1/125	−,−,−

[a] Three females per dilution were injected with the indicated dilutions of supernatants. Positives and negatives were scored as described under Materials and Methods.

VIF (obtained *in vitro*) up to 4 or 5 months after their blood meal lead to full vitellogenesis and egg-laying within a normal preoviposition period of 7 to 15 days (Aeschlimann 1968). Thus, a function of the VIF is to indicate the presence of sperm to the female reproductive system which can then start vitellogenesis and produce viable fertilized eggs.

TABLE III
Proteinase K Digestion of the Vitellogenesis Inducing Factor Found in *Ornithodoros moubata* Sperm Cell Incubation Medium

Digestion time (hr)	Dilutions tested	Response in bioassay[a]
Control without proteinase K, 6 hr at 30 C	1/1	+,+,+
	1/4	+,+,+
	1/16	+,+,+
	1/64	+,−,−
1	1/1	+,+,+
	1/4	+,+,+
	1/16	+,−,−
	1/64	−,−,−
3	1/1	+,+,−
	1/4	+,+,−
	1/16	−,−,−
	1/64	−,−,−
6	1/1	+,−,−
	1/4	−,−,−
	1/16	−,−,−
	1/64	−,−,−

[a] Three females per dilution were injected with the indicated dilutions of the reaction mixtures. Positives and negatives were scored as described under Materials and Methods.

Acknowledgment

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References

Aeschlimann, A. 1968. La ponte chez *Ornithodoros moubata* Murray (Ixodoidea, Argasidae). *Revue suisse de Zoologie* **75**, 1033–1039.

Balashov, Yu. S. 1972. Bloodsucking Ticks (Ixodoidea)—Vectors of Disease of Man and animals. *Miscellaneous Publications of the Entomological Society of America* **8**, 161–376.

Borut, S., and Feldman-Muhsam, B. 1976. A new observation on spermateleosis in ticks. *Journal of Parasitology* **62**, 318–320.

Diehl, P. A., Aeschlimann, A., and Obenchain, F. D. 1982. Tick reproduction: Oogenesis and oviposition. *In* "Physiology of Ticks" (F. D. Obenchain and R. Galun, eds.), pp. 277–380. Pergamon, Elmsford, N.Y.

Feldman-Muhsam, B., and Filshie, B. K. 1979. The ultrastructure of the prospermium of *Ornithodoros* ticks and its relation to sperm maturation and ca-

pacitation. *In* "The Spermatozoon" (D. W. Fawcett and J. M. Bedford, eds.), pp. 355–369. Urban & Schwarzenberg, Munich.

GEIGY, R., AND HERBIG, A. 1955. "Erreger und Uebertrager tropischer Krankheiten". (Recht und Gesellschaft, eds.). Basel.

GERMOND, J. E., AND AESCHLIMANN, A. 1977. Influence of copulation on vitellogenesis and egg-laying in *Ornithodoros moubata* MURRAY (Ixodoidea, Argasidae). *In* "Advances in Invertebrate Reproduction 1" (R. G. Adiyodi and Rita G. Adiyodi, eds.). Elsevier, Amsterdam/New York.

LAEMMLI, U. K., AND FAVRE, M. 1973. Maturation of the head of bacteriophage T4. I. DNA packaging events. *Journal of Molecular Biology* **80**, 575–599.

OLIVER, J. H. 1982. Tick reproduction: Sperm development and cytogenetics. *In* "Physiology of Ticks" (F. D. Obenchain and R. Galun, eds.), pp. 245–275. Pergamon, Elmsford, N.Y.

OLIVER, J. H., AND BRINTON, L. P. 1972. Cytogenetics of ticks (Acari: Ixodoidea). 7. Spermatogenesis in the pacific coast Tick, *Dermacentor occidentalis* Marx (Ixodidae). *Journal of Parasitology* **58**, 365–379.

OLIVER, J. H., AND SHEPHERD, J. 1981. Morphogenesis and activation of Tick sperm. *In* "Advances in Invertebrate Reproduction 2" (W. H. Clark, Jr. and T. S. Adams, eds.), pp. 243–251. Elsevier, Amsterdam/New York.

PINKERTON, A. M., HALL, J. D., AND SHEPHERD, J. 1982. Scanning electron microscopy of post-ejaculatory spermiogenesis in the tick *Ornithodoros moubata*. *Tissue and Cell* **14**, 785–797.

SHEPHERD, J., OLIVER, J. H., JR., AND HALL, J. D. 1982. A polypeptide from male accessory glands which triggers maturation of Tick spermatozoa. *International Journal of Invertebrate Reproduction* **5**, 129–137.

WÜEST, J., EL SAID, A., SWIDERSKI, Z., AND AESCHLIMANN, A. 1978. Morphology of the spermatid and spermatozoon of *Amblyomma hebraeum* KOCH (Acarina: Ixodidae). *Zeitschrift für Parasitenkunde* **55**, 91–99.