Studies on Eupyrene and Apyrene Spermatozoa in the Silkworm,
Bombyx mori L. (Lepidoptera: Bombycidae)

I. The Intratesticular Behaviour of the Spermatozoa at
Various Stages from the 5th-instar to the Adult

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Apyrene sperm bundles showed their first appearance from the testicular follicle into the vas efferens at 144 hours after pupation. The bundles were separated when they passed through the basement membrane. At 192 hours after pupation the majority of the separate apyrene spermatozoa was observed in the vas efferens. On the other hand, the eupyrene sperm bundles first appeared from the follicle into the vas efferens at 192 hours after pupation. At 240 hours after pupation (the day before emergence) the majority was observed in the vas efferens. Maintaining their bundle in the intact state, those eupyrene sperm bundles passed through the basement membrane with their tails ahead and were mixed with the separate apyrene spermatozoa.

When the apyrene sperm bundles appeared from the follicle, the tissue of the basement membrane became rough in appearance and when the majority of the apyrene spermatozoa was accumulated in the vas efferens, the tissue began to recover its original state. Before the recovering of the tissue was completed, the eupyrene sperm bundles began to appear in the vas efferens and the tissue resumed a remarkable rough appearance again. From the above observation, it was suggested that the apyrene spermatozoa played an important role in facilitating the passage of the eupyrene sperm bundles through the basement membrane.

INTRODUCTION

In the silkworm, eupyrene and apyrene spermatozoa were observed for the first time by TOYAMA (1894), and thereafter, the spermatozoa of the types in the same material were confirmed by several investigators. With regard to other species of Lepidoptera, as in the case of the silkworm, both types of spermatozoa were observed by many investigators since MEVES (1903) made his description.

In the silkworm, eupyrene and apyrene spermatozoa were formed in the testicular follicle, but as to whether apyrene spermatozoa appeared from the follicle or not, different opinions were proposed. (TSUKAGUCHI and KUROTSU, 1922; MACHIDA, 1929; ÔMURA, 1936; IRIKI, 1941; SUZUKI and ÔMURA, 1951; SADO, 1963). At present time, it has become definite that apyrene spermatozoa appear from the follicle in a similar manner to eupyrene spermatozoa, and furthermore, both types of spermatozoa were ejaculated into the copulatory pouch after mating and migrated into the seminal receptacle. These phenomena were also observed in other species of Lepidoptera

(RIEMANN, 1970; PHILLIPS, 1971; RIEMANN and THORSON, 1971).

The present paper deals with the morphological change and movement of the spermatozoa in the follicle by the histological observations of the testes at various stages from the 5th instar to the adult.

MATERIALS AND METHODS

The material used for the research was the hybrid J 131 $\times$ C 131 and was bred under the condition of constant temperature (25°C) and humidity (R. H. 75%). The testes at the various stages from the 5th instar to the adult was fixed with Bouin's solution sectioned in 5- μ thickness and stained with Delafield's haematoxylin and eosin.

RESULTS

From the 3rd day of the 5th instar to the prepupal stage

In the larva on the 3rd day of the 5th instar, the follicle was mainly occupied by the cytocyts in which spermatogenesis was thriving. A small number of eupyrene sperm bundles which located above the basement membrane of the follicle were stained in light blue. After the 5th day of the 5th instar, these cytocyts and bundles were observed at a high incidence. During the period from the end of the 5th instar to the day before pupation, a large number of cytocyts developed into eupyrene sperm bundles and the follicle were mainly occupied by these bundles. Most of them were stained in light blue but the several eupyrene sperm bundles which located above the basement membrane were stained in light red or red.

Immediately after pupation

Only a small number of apyrene sperm bundles which were stained in light blue appeared around the apical cell for the first time.

24 hours after pupation

The eupyrene sperm bundles which located in the lower part of the follicle were stained in light red or red. The spermatogonia around the apical cell gradually developed into apyrene sperm bundles, which were seen spreading around the apical cell and along the wall of the blind end (Fig. 1).

48 hours after pupation

The eupyrene sperm bundles which occupied the lower one-third part of the area of the eupyrene sperm bundles in the follicle were stained in light red or red. The rest bundles were stained in light blue. As apyrene sperm bundles developed from the cytocyts in the upper part of the follicle, they moved along the lateral wall toward the open end to surround the eupyrene sperm bundles which occupied the central part of the follicle.

72 hours after pupation

A small number of apyrene sperm bundles along the lateral wall migrated toward the proximal part of the lateral wall and the basement membrane.

96 hours after pupation

At this stage the space of the eupyrene sperm bundles which were stained in light red or red increased up to a lower half of the follicle. As apyrene sperm bundles which developed from the cytocyts in the upper part of the follicle spread toward the open end, the entire eupyrene sperm bundles in the follicle were pushed toward the open

end. As a result, the space which was occupied by the eupyrene sperm bundles gradually became narrower. A small number of apyrene sperm bundles in the proximal part of the lateral wall and above the basement membrane ejected small granule-like nuclei. These bundles took a rough appearance and were stained in light brown.

120 hours after pupation

The eupyrene sperm bundles in the follicle showed an irregular orientation.

144 hours after pupation

The space which was occupied by the eupyrene sperm bundles in the follicle narrowed and became two-thirds from the open end due to the increase of apyrene sperm bundles, and all of these eupyrene sperm bundles were stained in light red or red. A large number of apyrene sperm bundles along the lateral wall cast off small granule-like nuclei slightly above the proximal part (Fig. 2). These bundles assumed a rough appearance and were stained in light brown. They began to congregate at the proximal part of the lateral wall and above the basement membrane, taking an S form or an irregular and twisted shape (Figs 3 and 4). Thereafter, a part of the apyrene sperm bundles above the basement membrane migrated into the vas efferens for the first time (Fig. 7-A). They shed their sheaths in the follicle and were separated into spermatozoa when they passed through the basement membrane.

150 hours after pupation

The space which was occupied by the eupyrene sperm bundles showed a successive narrowing. The eupyrene sperm bundles in the follicle began to descend toward the open end with their tails ahead. The descending movement of the apyrene sperm bundles along the lateral wall completed. Apyrene sperm bundles which had already ejected small granule-like nuclei showed a remarkable congregation above the basement membrane and a considerable amount of the apyrene spermatozoa appeared from the follicle into the vas efferens (Fig. 5). A large number of sheaths from the bundles were observed above the basement membrane of the follicle. At this stage the histological appearance of the basement membrane was perfect but the nuclei in the cells of the membrane became flatter and began to migrate toward the lower part of the cell.

168 hours after pupation

The eupyrene sperm bundles, all of which were now stained in light red or red, occupied only a half space or less of the follicle from the open end and thereafter did not change till the day before emergence. Several eupyrene sperm bundles intruded with their tails ahead into the region above the basement membrane which was occupied by apyrene sperm bundles without nuclei and by their cast sheaths (Fig. 6). The apyrene spermatozoa in the vas efferens decreased temporarily because these spermatozoa migrated into the seminal vesicle. The tissue of the basement membrane took a rough appearance and a part of the apyrene sperm bundles which had ejected small granule-like nuclei were observed in the tissue (Fig. 6). The nuclei in the cells of the membrane tissue were still seen in the lower part and assumed a triangular or indefinite shape (Fig. 6).

192 hours after pupation

Though eupyrene sperm bundles gradually descended toward the basement membrane, the space which was occupied by the eupyrene sperm bundles was not changed, and accordingly, density of the bundles decreased in the upper part of this space, while in the region above the basement membrane it became rather high. Those bundles at this stage showed such a definite orientation as their tails directed toward the open

end of the follicle (Fig. 8). The upper half space from the blind end of the follicle contained apyrene sperm bundles which still kept small granule-like nuclei. The majority of the non-nucleated apyrene sperm bundles which had been observed above the basement membrane at the previous stage migrated into the vas efferens. Eupyrene sperm bundles also shed their sheaths and left them in the follicle and appeared for the first time from the follicle into the vas efferens through the basement membrane with their tails ahead, as described by ÔMURA (1936). During this stage, a large number of apyrene spermatozoa were mixed with a small number of eupyrene sperm bundles in the vas efferens (Fig. 7-B). Many eupyrene sperm bundles and the sheaths from the eupyrene and apyrene sperm bundles were observed above the basement membrane. Only a small number of apyrene sperm bundles which ejected small granule-like nuclei were observed above the basement membrane but a considerable amount of them were observed at the proximal part of the lateral wall, and this state was maintained till the time of emergence. When the non-nucleated apyrene sperm bundles which intruded into the tissue of the basement membrane migrated into the vas efferens, their sheaths were left in the tissue (Fig. 9).

216 hours after pupation

A large number of eupyrene sperm bundles were observed above the basement membrane and gradually migrated into the vas efferens. The nuclei in the cells of tissue of the basement membrane took a round and elliptical shape and the tissue began to recover to its original state.

240 hours after pupation

A large number of eupyrene sperm bundles migrated into the vas efferens and were mixed with apyrene spermatozoa which had migrated earlier than eupyrene sperm bundles (Figs. 7-C, 10 and 11). Accordingly, eupyrene sperm bundles in the follicle decreased and a large amount of the sheaths of the bundles were observed above the basement membrane. At this stage, the appearance of eupyrene sperm bundles and apyrene spermatozoa from the follicle into the vas efferens in the pupal stage was completed. When eupyrene sperm bundles began to appear from the follicle the tissue of the basement membrane took a rough appearance and the nuclei of the cells of the tissue became a triangular and elliptical shape again. The sheaths of the bundles were still observed in the tissue of the basement membrane.

Immediately after emergence

At the time of emergence, the testes shrunk and the space which was occupied by eupyrene sperm bundles narrowed to one-third of the follicle or less from the open end side. The eupyrene sperm bundles in this space became slightly dense. A part of them were seen scattered between the numerous sheaths of the bundles above the basement membrane. In some cases, numerous sheaths of the bundles alone were observed above the basement membrane (Fig. 12). Cytoplasm of the sheaths adjacent to the basement membrane was substantial, but as the distance between the site of the sheaths and the basement membrane became farther, the cytoplasm appeared rough. In other words, the sheaths which were cast off at the earlier stages took a rough appearance. The numerous sheaths left in the follicle looked like a mesh as a whole, and the nuclei were distributed in the thin cytoplasm of the mesh. The tissue of the basement membrane began to resume its original state again and the nuclei of an elliptical shape were observed in the lower part of the cell. At the same time the sheaths which intruded into the tissue became indistinct and were absorbed by the tissue (Fig.

12). The majority of the cytocyts in the upper part of the follicle became apyrene sperm bundles and these bundles occupied the space of upper two-third of the follicle. A very few groups of them were noted to eject small granule-like nuclei (Fig. 13). Furthermore, a considerable amount of apyrene sperm bundles without nuclei were observed at the proximal part of the lateral wall. Only a few spermatogonia were seen around the apical cell.

DISCUSSION

The appearance of the spermatozoa from the testicular follicle into the vas efferens

Existence of the eupyrene and apyrene spermatozoa was recognized by many investigators in Lepidoptera since MEVES (1903) set forth his description. Furthermore, the fact that apyrene spermatozoa get out from the follicle as well as eupyrene spermatozoa was confirmed in *Trichoplusia ni* of Lepidoptera (RIEMANN, 1970) and in *Anagasta kuhniella* of Lepidoptera (RIEMANN and THORSON, 1971; RIEMANN et al., 1974). Especially in the latter, it was observed that apyrene spermatozoa got out from the follicle earlier than eupyrene spermatozoa. In the silkworm, eupyrene and apyrene spermatozoa were observed by several investigators since TOYAMA (1894) and MEVES (1903). MACHIDA (1929) described that in an old pupa apyrene spermatozoa generally degenerated in the distal portion of the follicle and ÔMURA (1936) stated that apyrene spermatozoa could rarely be observed outside the follicle. On the other hand, TSUKAGUCHI and KUROTSU (1922), and IRIKI (1941) obtained the conflicting result that both types of spermatozoa were observed in the post-testicular organs of the male adult and in the internal reproductive organs of the female adult after mating. SUZUKI and ÔMURA (1951) reported that a large number of apyrene spermatozoa, as well as eupyrene spermatozoa, were observed *in vitro* in the seminal vesicle of the abnormal adult of the oligospermy strain and in the copulatory pouch and seminal receptacle of the normal female adults mated with these abnormal males. On the other hand, SADO (1963) found no apyrene spermatozoa in the seminal vesicle or in any reproductive organs of mated females, and supported the earlier observation by MACHIDA (1929) and ÔMURA (1936).

It is considered that these two conflicting results on both types of spermatozoa may be caused by the following reason: 1. The morphological change and behaviour of both types of sperm bundles in the follicle were not studied clearly. 2. It was often difficult to distinguish the both types of spermatozoa in the post-testicular organs of the male and reproductive organs of the mated females by paraffin sectioning method alone.

In the present study each characteristic morphological change and behaviour of the both types of the sperm bundles in the follicle was observed at the stages from 5th instar to the adult by paraffin sectioning method and it was concluded that apyrene sperm bundles appeared into the vas efferens from the follicle earlier than eupyrene sperm bundles. According to the author's observations on both types of spermatozoa in the seminal vesicle, eupyrene sperm bundles and separated apyrene spermatozoa could be distinguished *in vitro* by a phase contrast microscope, though it was often difficult to tell the difference of them by paraffin sectioning method. It was necessary, therefore, to make observations *in vitro* in addition to the paraffin sectioning method in order to distinguish the both types of spermatozoa in the seminal vesicle. The author

confirmed that apyrene sperm bundles appeared from the follicle in a separate from when they passed through the basement membrane, and thereafter, they migrated into the seminal vesicle through the vas deferens earlier than eupyrene sperm bundles.

The behaviour of the spermatozoa in the follicle

In *Chortophaga viridifasciata* (Orthoptera), PAYNE (1933) made an interesting observation *in vitro* on the appearance of the spermatozoa from the testes. He discovered that the spermatozoa moved rather regularly within the follicle of the testes when ascending, crossing and descending, and then appeared in the vas deferens. He explained that the descending posture toward the basement membrane was due to gravity. In *Cimex lectularis* (Heteroptera), DAVIS (1956) described that the head of the sperm bundle at the upper part of the follicle was directed toward the blind end in the early spermatid stage. As the bundle continued to elongate, the apex of the bundle turned and it began to move toward the vas efferens. When the bundle reached the vas efferens it completely turned around and the spermatozoa were fully matured. This process of elongation and turning of the sperm bundles was similar to that described in the *Leptocoris trivittatus* (Hemiptera) by PAYNE (1934) who suggested that it may be of general occurrence in Hemiptera. In *Anagasta kühniella*, RIEMANN et al. (1974) stated that apyrene sperm bundles got out from the follicle earlier than eupyrene sperm bundles and eupyrene sperm bundles appeared from the follicle with their tails ahead, but those authors did not describe as to behaviour of the both types of sperm bundles in the follicle. In the silkworm, ÔMURA (1963) reported that gravity had no effect on the descending posture of the eupyrene sperm bundles. The eupyrene sperm bundles which congregated on the basement membrane kept their heads close but not attached to it. Then, remaining their head at the same position, the bundles were turned for 180° only by the movement of the tail part and they migrated into the vas efferens through the basement membrane with their tails ahead.

According to the present observation in the silkworm, apyrene sperm bundles began to get out from the follicle at 144 hours after pupation and were separated when they passed through the basement membrane. In the apyrene sperm bundles or apyrene spermatozoa, it could not be distinguished which side was the head and which side was the tail. The apyrene sperm bundles ejected small granule-like nuclei in the follicle, but the significance of the ejection has not been cleared. As to the ejection of small granule-like nuclei, a similar observation was reported by ZYLBERBERG (1969) in *Pieris brassicae* (Lepidoptera). On the other hand, the eupyrene sperm bundles in the follicle showed irregular orientation at 120 hours after pupation. At 150 hours after pupation they began to descend toward the open end with their tails ahead. At 192 hours after pupation the eupyrene sperm bundles moved down and congregated above the open end. Then, maintaining their orientation, the sperm bundles got out from the follicle through the basement membrane. The bending and turning of the eupyrene sperm bundles as described by ÔMURA (1936) were not observed in the present study.

The period when the sperm bundles appeared from the follicle was the late pupal or preimaginal stage in *Prinoplus reticularis* of Coleoptera (EDWARDS, 1961). In the silkworm, eupyrene sperm bundles began to appear from the follicle 3~4 days before emergence (ÔMURA, 1936). According to the present observation, apyrene sperm bundles began to get out from the follicle at the stage about 5 days before emergence (144 hours after pupation) and eupyrene sperm bundles began to appear from the follicle

at the stage about 3 days prior to emergence (192 hours after pupation). The interval of the appearance of apyrene sperm bundles from the follicle and that of the eupyrene sperm bundles was 48 hours. It was not clear, however, by the morphological observation of the bundles in the follicle alone whether the eupyrene sperm bundles remained in the follicle in order to prepare for the appearance or not.

Passage of the sperm bundles through the basement membrane

The basement membrane which formed a boundary between the follicle and the vas efferens were observed in the scarabeid beetle (VIRKKI, 1956), *Prinoplus reticularis* (EDWARDS, 1961), and *Anagasta kühniella* (RIEMANN et al., 1974), but the change in this tissue at the time of the passage of the sperm bundles was not reported. IKEDA (1913) also concluded that the spermatozoa flowed out from the follicle through a pathway which appeared at a certain position in the basement membrane, though ÔMURA (1936) denied the theory of IKEDA. He observed no special pathway for the sperm bundles in the basement membrane before or after the sperm bundles appeared from the follicle and concluded that each bundle could pass through the basement membrane in a particular and complex manner, boring its own pathway by its own action. According to the present observation in the silkworm, when the apyrene sperm bundles appeared from the follicle, the tissue of the basement membrane showed a rough appearance, and when the majority of the bundles completed their passage, the tissue began to resume its original state. The eupyrene sperm bundles appeared in the vas efferens before the tissue finished its recovery and the tissue became remarkably rough again. The present observation suggested that the apyrene spermatozoa played an important role in facilitating the passage of the eupyrene sperm bundles through the basement membrane.

Separation of the sperm bundles

In *Tricoplusia ni* (RIEMANN, 1970) and *Anagasta kühniella* (RIEMANN et al., 1974), the apyrene sperm bundles separated immediately after appearing from the follicle and the eupyrene sperm bundles separated in the reproductive organs of the mated females. In the silkworm, the apyrene sperm bundles separated in the course of passing through the basement membrane and the eupyrene sperm bundles separated in the copulatory pouch of the mated females. In the silkworm, the separation of the eupyrene sperm bundles which appeared from the testes occurred by chance (IKEDA, 1913) or mechanically (ÔMURA, 1936). However, it was considered that these bundles described in his papers seemed to correspond to the apyrene sperm bundles in the present observations. Furthermore, the separation of the apyrene sperm bundles did not seem to occur by chance or mechanically, though the factor that contributes to the separation of the bundles is not clear.

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REFERENCES

- DAVIS, N. T. (1956) The morphology and functional anatomy of the male and female reproductive systems of *Cimex lectularius* L. (Heteroptera, Cimicidae). *Ann. ent. Soc. Am.* **49**: 466-493.

- EDWARDS, J. S. (1961) On the reproduction of *Prinoplus reticularis* (Coleoptera, Cerambycidae), with general remarks on reproduction in the Cerambycidae. *Quart. J. micr. Sci.* **102**: 519-529.
- IKEDA, E. (1913) *Anatomical and Physiological Researches on the Silkworm*. Meibundo Press, Tokyo, 1513 pp. (in Japanese).
- IRIKI, S. (1941) On the function of apyrene spermatozoa of the silkworm. *Zool. Mag.* **53**: 123-124 (in Japanese).
- MACHIDA, J. (1929) Fine experimentelle Untersuchung über die apyrene Spermatozoen des Seiden-spinners *Bombyx mori* L. *Z. Zellforsch. mikro. Anat.* **9**: 466-510.
- MEVES, F. (1903) Ueber oligopyrene und apyrene Spermien und über ihre Entstehung, nach Beobach-tungen an *Paludina* und *Pygaera*. *Arch. mikro. Anat.* **61**: 1-82.
- Ômura, S. (1936) Studies on the reproductive system of the male of *Bombyx mori*. I. Structure of the testis and the intratesticular behaviour of the spermatozoa. *J. Fac. Agric. Hokkaido Imp. Univ.* **38**: 151-181.
- PAYNE, M. A. (1933) The structure of the testis and movement of sperms in *Chortophage viridifasciata* as demonstrated by intravital technique. *J. Morphol.* **54**: 321-346.
- PAYNE, M. A. (1934) Intravital studies of the hemipteran, *Leptocoris trivittatus*. A description of the male reproductive organs and the aggregation and turning of the sperms. *J. Morphol.* **56**: 513-531.
- PHILLIPS, D. M. (1971) Morphogenesis of the Iacinate appendages of lepidopteran spermatozoa. *J. Ultrastruct. Res.* **34**: 567-585.
- RIEMANN, J. G. (1970) Metamorphosis of sperm of the cabbage looper, *Trichoplusia ni*, during passage from the testes to the spermatheca. In *Comparative Spermatology* (ed. B. BACETTI) pp. 321-331 Academic Press, New York.
- RIEMANN, J. G. and B. J. THORSON (1971) Sperm maturation in the male and female genital tracts of *Anagasta kühniella* (Lepidoptera: Pyralidae). *Int. J. Insect Morphol. and Embryol.* **1**: 11-19.
- RIEMANN, J. G., B. J. THORSON and R. L. RUVD (1974) Daily cycle of release of sperm from the testes of the Mediterranean flour moth. *J. Insect Physiol.* **20**: 195-207.
- SADO, T. (1963) Spermatogenesis of the silkworm and its bearing on radiation induced sterility. I. *J. Fac. Agric. Kyushu Univ.* **12**: 359-386.
- SUZUKI, K. and S. ÔMURA (1951) Inheritance of non-fertilization phenomena in the silkworm. II. On the oligospermy. *Bull. Sericul. Exp. Stn* **13**: 347-366 (in Japanese).
- TOYAMA, K. (1894) On the spermatogenesis of the silkworm. *Bull. Coll. Agric. Tokyo Imp. Univ.* **2**: 125-188.
- TSUKAGUCHI, R. and T. KUROTSU (1922) On the apyrene spermatozoa of the Lepidoptera. *Zool. Mag.* **34**: 310-317 (in Japanese).
- VIRKKI, N. (1956) Zur Kenntnis der postmeiotischen Ereignisse der Samenentwicklung bei den Skarä-baiden (Coleoptera). *Z. Zellforsch.* **44**: 644-665.
- ZYLBERBERG, L. (1969) Contribution à l'étude de la double spermatogenèse chez in Lépidoptèra (*Pieris brassicae* L. Pieridae). *Annls Sci. nat. Zool. Ser.* **12**, **11**: 569-626.

Fig. 1. ~Fig. 6.

Fig. 7. The process of the appearance of eupyrene and apyrene sperm bundles from the follicle to the vas efferens. A, 144 hours after pupation; B, 192 hours after pupation; C, 240 hours after pupation.

Fig. 1. 24 hours after pupation. Eupyrene sperm bundles and apyrene sperm bundles before ejecting small granule-like nuclei. $\times 460$.

Fig. 2. 144 hours after pupation. Apyrene sperm bundles ejecting small granule-like nuclei are shown slightly above the proximal part of the lateral wall. $\times 460$.

Fig. 3. 144 hours after pupation. Apyrene sperm bundles after ejecting small granule-like nuclei congregate at the proximal part of the lateral wall. $\times 255$.

Fig. 4. 144 hours after pupation. Apyrene sperm bundles after ejecting small granule-like nuclei congregate above the basement membrane, taking an S form or an irregular and twisted form. $\times 545$.

Fig. 5. 150 hours after pupation. Apyrene sperm bundles after ejecting small granule-like nuclei appear in the vas efferens from the follicle. A large number of apyrene spermatozoa are observed in the vas efferens. $\times 460$.

Fig. 6. 168 hours after pupation. Eupyrene sperm bundles intrude into the space above the basement membrane which is occupied by apyrene sperm bundles without nuclei and their sheath. Apyrene sperm bundles are also seen in the tissue of the basement membrane. Nuclei in the cell of the tissue show a triangular or indefinite shape. $\times 460$.

Fig. 8. ~Fig. 13.

Fig. 8. 192 hours after pupation. Orientation of eupyrene sperm bundles in the follicle. The bundles and the tails of the bundles direct to the basement membrane. $\times 160$.

Fig. 9. 192 hours after pupation. Eupyrene sperm bundles intrude into the space above the basement membrane which is mainly occupied by the sheaths of sperm bundles. Sheaths of sperm bundles are shown in the tissue of the basement membrane. $\times 460$.

Fig. 10. 240 hours after pupation. Eupyrene sperm bundles appearing from the follicle. $\times 460$.

Fig. 11. 240 hours after pupation. Eupyrene sperm bundles and apyrene spermatozoa in the vas efferens. $\times 120$.

Fig. 12. Immediately after emergence. Numerous sheaths of the bundles alone are seen above the basement membrane. Tissue of the basement membrane recover its original state again. Sheaths of the bundles are absorbed by the tissue. $\times 460$.

Fig. 13. Immediately after emergence. Apyrene sperm bundles before or after ejecting small granule-like nuclei. $\times 235$.

Abbreviation: ab1, apyrene sperm bundle before ejecting small granule-like nuclei; ab2, apyrene sperm bundle ejecting small granule-like nuclei; ab3, apyrene sperm bundle after ejecting small granule-like nuclei; ab4, apyrene sperm bundle without small granule-like nuclei in the tissue of the basement membrane; ac, apical cell; as apyrene spermatozoa; bm, basement membrane; eb, eupyrene sperm bundle; n, nucleus of the basement membrane cell; sh, sheath of the sperm bundle; tf, testicular follicle; ve, vas efferens.