

SPERMATOGENESIS AND OOGENESIS IN MAYFLIES (*EPHEMEROPTERA*)

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Although already BRANDT (1878) and PALMÉN (1884) described the ovarioles of *Ephemeroptera* as panoistic, only several works discuss the reproductive organs and spermatogenesis and oogenesis. The first more detailed data were published by NEEDHAM, TRAVER & HSU (1935). The reproductive organs were studied to the greatest detail by BRINCK (1957) in 8 genera. He also described the descent of the eggs into the oviduct and of the sperms into the vas deferens. The reproductive organs in *Coloburiscus humeralis* (WALKER) were described by WISELY (1965). The knowledge of the eggs of *Ephemeroptera* is of course extensive. Chorion is described in the majority of known genera (DEGRANGE, 1960, KOSS, 1968) and fecundity is also known in many genera (DEGRANGE, 1960, CLIFFORD & BOERGER, 1974). The structure or more precisely the ultrastructure of the sperms was studied only in one species (BACCETTI, DALLAI & GIUSTI, 1969).

Material and Methods

Spermatogenesis and oogenesis were studied on larvae, subimagos and imagoes of six species. These are *Cloeon dipterum* (L.), *Oligoneuriella rhenana* (IMHOFF), *Ecdyonurus torrentis* KIMMINS, *Caenis robusta* EATON, *Paraleptophlebia submarginata* (STEPHENS) and *Ephemerella danica* (MÜLL.). These species represent different phylogenetic branches and different morphological and ecological types. All material studied originates from localities in Central Bohemia. Testes and ovaries were fixed in BOUIN's fluid and CARNOY's fluid, then transferred through alcohol and methylbenzoate and embedded into paraplast. The sections were cut on a microtome to thickness of 5 μ m. The nuclei in the sections were stained with HARRIS haematoxylin and cytoplasm counter-stained with eosin. The modified PAPPENHEIM's method was also used for staining sections, smears and squashes. The fast green stain was used for staining of native preparations for examination in phase, anoptical and interference contrast microscope.

Results

Spermatogenesis takes place in follicles of the testis (Pl. I, Fig. 3) from early larval instars. In younger larvae that is in larvae approximately up to the tenth instar, the whole of the follicle is filled by germarium with spermatogonia. Spermatogonia are of spherical shape about 3-5 μ m in size with

a stainable nucleus and with evident nucleolus (Pl. I, Fig. 1). A single follicle contains several tens of spermatogonia. The VERNER'S cell is not developed, the cells with a nutritional function are dispersed among spermatogonia. In even younger larvae cysts are being formed (Pl. I, Figs. 2, 4) and in addition to germarium the formation of a zone of growth can be observed. In "winter" species (LANDA, 1968) *E. torrentis*, *P. submarginata* at the beginning of autumn always only one spermatogonium is enclosed in one cyst. Approximately in the middle of the larval stage the spermatogonia in cysts divide into spermatocytes, the cysts enlarge and at the same time the whole of the follicle increases in size as well. Spermatocytes are about 6–9 μm in size, the nuclei are smaller, less intensively stainable but with well-stainable nucleoli (Pl. II, Fig. 5). It is very difficult to distinguish by staining of the nucleus and by the amount of cytoplasm between spermatocytes I and II. The meiosis takes place in "winter" species during winter. In this period germarium disappears and the zone of growth is reduced to the apical part of the follicle. The rest of the follicle includes the zone of maturation with cysts containing about 8–16 spermatocytes. In older larvae that is in larvae approximately from the tenth instar with fully developed larval characters and in "winter" species at the beginning of spring—in these older larvae spermatids begin to appear in cysts of the basal part of the follicle. Spermatids are about 4 μm in size, with a large intensively stainable nucleus, without evident nucleolus and with hardly any cytoplasm (Pl. II, Fig. 6). In this period the cysts usually desintegrate, but the spermatids remain arranged in groups. But the cysts can also desintegrate earlier or later since sometimes even free spermatocytes in follicle or on the contrary praesperms in cysts can be found. During desintegration of cysts the zone of transformation is formed in the base of the follicle and the rest of the follicle is filled with the zone of maturation. The other zones disappear. Already several instars before moulting (in "winter" species in spring) the first praesperms appear. These are similar to spermatids in shape, but with a foundation of a tail (Pl. II, Fig. 7). The tail is usually coiled up into a little ball, but sometimes it is not visible at all (*P. submarginata*). These praesperms elongate, the tail grows longer and praesperms transform into sperms (Pl. II, Fig. 8). Spermatocytes in the follicle gradually fade away and the whole of follicle is filled up with the zone of transformation. The mature sperms descend into vas deferens and the seminal vesicle (Pl. III, Figs. 9, 10, 11). In the last instar the follicles of the testis are converted into hardly discernible membranous formations and nearly all sperms are already in the seminal vesicle or in the vas deferens. In vas deferens the rest of the praesperms can usually be found. Sperms of *Ephemeroptera* are always of two types (Pl. III, Fig. 12). On the one hand there are the more numerous, smaller and well-stainable sperms, on the other hand the less numerous, of double size and less well-stainable types. But the both types of sperms are of the same shape. Sperms are rod shaped (*C. dipterum*, *C. robusta*, *E. danica*), spindle shaped (*O. rhenana*, *E. torrentis*), spherical and in the optical microscope without any discernible tail (*P. submarginata*). They are 3–8 μm in size (up to 15 μm in the less well-stainable type) and about 2–4 (6–7) μm wide.

Oogenesis takes part in the ovarioles of ovarium (Pl. V, Fig. 17) already from the earliest larval instars. In younger larvae (in "winter" species at the end of summer and at the beginning of autumn) the ovarioles are still short, filled up completely by germarium with oogonia. The oogonia almost do not differ in size and shape from spermatogonia. In the "winter" species the division of oogonia into oocytes takes part still in autumn. The oocytes are formed first in the basal part of the ovariole (Pl. IV, Fig. 13). At the same time with the formation of the oocytes the whole germarium elongates (Pl. V, Figs. 18, 19). The shape of the oocytes is already similar to the future egg. Oocytes (Pl. IV, Fig. 14) are about 9–12 μm in size, spherical (*O. rhenana*, *E. torrentis*), slightly elongated (*E. danica*) or oval (*P. submarginata*). The nucleus is less intensively stainable than that of the oogonium but with an intensively stainable nucleolus (*macula germinativa*). In germarium among oocytes there are also the follicular cells which are considerably smaller. In about the middle of the larval stage only a narrow layer of oogonia remains in the apical part of ovariole. In this period (in the "winter" species at the beginning of winter) 2–4 oocytes nearest to the oviduct increase in size. After reaching double the size than the other oocytes, they are surrounded by the follicular cells (Pl. IV, Fig. 16). At the beginning this follicular epithelium has two or three layers, later only a single layer. Oocytes surrounded by follicular cells align themselves linearly at the basis of the ovariole (Pl. V, Fig. 20) and so constitute the vitellarium. Ovariole elongates. During this period the meiotic division takes place. The

nucleolus disappears and the nucleus changes into the characteristic formation (*vesicula germinativa*) (Pl. IV, Fig. 15). After meiosis is completed, oocytes have a very weakly stainable nucleus located in the central position without a visible nucleolus and with a large amount of cytoplasm. When the size of the increasing oocytes reaches half the size of the mature egg, vitellogenesis begins (that is in older larvae and in "winter" species at the beginning of spring). Yolk granules gradually superimpose on the nucleus, the oocytes continue growing (Pl. VI, Figs. 21, 22) and the vitellarium elongates considerably. Each ovariole usually contains 5-7 oocytes (*P. submarginata*, *E. torrentis*), even 9 oocytes (*E. danica*, *C. robusta*) or only 1-2 oocytes (*C. dipterum*). The oocytes situated nearest to the oviduct mature first. Here the chorion is formed by the secretion of follicular cells which later degenerate (Pl. VI, Fig. 23). About 3-4 instars before moulting to subimago the mature eggs begin to descend into the oviducts. The mechanism of the descent of the eggs is similar to the turning inside out of a finger of a rubber glove which is inflated at the same time. The vitellarium with mature eggs is pulled into the dilating oviduct. Germarium and about 2-3 enlarged oocytes are not pulled inside the oviduct, but change into a stringy membranous formation with a well apparent terminal filament, which later (in the last instar) disappears entirely. Both oocytes in germarium and the youngest oocytes in vitellarium degenerate and are resorbed (Pl. VI, Fig. 24). In larvae of the last instar all eggs are already in the oviduct. The eggs of the above mentioned species were described by DEGRANGE (1960). In subimagos of some species the shape of eggs changes. These changes take place only in those species in which the subimaginal stage is longer than 1 day. The most intensive changes are in *P. submarginata* (Pl. VII, Figs. 25-28). Here in the equatorial region grooves appear and the shape of eggs is greatly deformed. The changes of the shape of eggs can be also found in *E. danica*. Here the originally oval egg changes into irregular polyhedron. Less intensive changes can also be found in *E. torrentis*. In *C. dipterum* the eggs do not descend into the oviduct although the oviduct dilates. They remain in the membranous ovarioles both in the subimago and imago. The germarium degenerates in the same way as in the other species.

Discussion and Conclusions

The obtained results conform to hitherto known literary data concerning anatomical and histological structure of the reproductive organs. The changes of shape of eggs in subimago and imago described by BENGTSOON (1913) and DEGRANGE (1960) in *E. danica* were observed also in other species. The study of spermatogenesis and oogenesis in six model species brought important results not only for the study of *Ephemeroptera*, but also for other general problems. The spermatogenesis in *Ephemeroptera* takes place evidently only in one wave. In the follicles of the testis always only one type of cells predominates. In one follicle there are never found spermatogonia and spermatids simultaneously with the sperms as it is usual in insects that multiply several times in adult age. Otherwise spermatogenesis takes place in the usual way. The sperms of *Ephemeroptera* are worth our attention. Although they were studied only in six species, considerable differences in shape and size were observed. The sperms of *P. submarginata* are entirely atypical. In the optical microscope the tail is not discernible. The sperms of *Ephemeroptera* are always of two types. The larger and the less numerous sperms are probably of apyrenne type and serve for the nourishment of sperms with nuclei. The oogenesis also takes place in one wave. In the ovariole oogonia are never found simultaneously with mature eggs. Although the oocytes in ovarioles mature gradually as in insects that lay eggs several times, only several of the oldest oocytes mature and the other oocytes including the not yet enlarged ones degenerate in the germarium. Otherwise oogenesis takes place in the same way as it is usual in insects with panoistic ovarioles. As an unusual fact remain the changes of shape of the eggs that can be observed in some species during the stage of subimago. Out of the studied species these changes are most evident in *P. submarginata*. There are two possible explanations of this phenomenon:

1. The eggs lose water during the subimaginal stage so that the weight of the future imago would be lower and the flight abilities be better. The results obtained by weighing subimagos of different

age give evidence for this hypothesis. While females lose as much as 25% of weight during the subimaginal stage, the males only 15–20%. Since all the organs of males and females are developed in the same way and the loss of water from the rest of the testis is minimal, the reduction of weight must originate in the eggs.

2. The eggs are deformed only in a passive way so that they can arrange themselves in the oviduct economically that means that each egg occupies a minimal area. The fact that the eggs acquire regular shape immediately after leaving the oviduct and after eggs-laying, gives evidence for this hypothesis.

Another specific sign of spermatogenesis and oogenesis of *Ephemeroptera* is that these are concentrated in the larval stage. The first sperms and the first eggs develop already in older larvae and in larvae of the last instar almost all sexual cells are in the stage of mature sperms and eggs. In the last instar extensive degeneration of testicular follicles and of ovarioles can even be observed. This degeneration is a rare phenomenon in the other insects. During the subimaginal stage already only slight changes take place (in species where this stage lasts longer, the descent of sperms and eggs into the seminal vesicle and the oviduct is being terminated). It is generally regarded as a fact that the shorter the subimago stage the earlier the spermatogenesis and oogenesis is terminated. Since in the ontogenetic development of *Ephemeroptera* the trofic stage (larva) and the reproductive stage (subimago and imago that do not receive nourishment) alternate, the energetically demanding processes of spermatogenesis and oogenesis were shifted to no other than the larval that is the trofic stage.

SUMMARY

Spermatogenesis and oogenesis in mayflies (Ephemeroptera)

Spermatogenesis and oogenesis in the genera *Cloeon*, *Oligoneuriella*, *Ecdyonurus*, *Caenis*, *Paraleptophlebia* and *Ephemer* have been studied. Testicular follicles of the first instar larvae contain only spermatogonia. In young larvae (i. e. to about 10th instar) the spermatogonia are encysted and dividing into spermatocytes. In older larvae (over 10th instar) the cysts disintegrate, and the spermatocytes which have undergone reduction division develop into spermatids. Spermateliosis takes place only in the last instars. Mature sperms descend into the seminal vesicle and the testicular follicles degenerate. The germaria of ovarioles of the first instar female larvae contain only oogonia. In young larvae the oogonia gradually develop into oocytes which in older larvae increase in size, are surrounded with follicular cells and form a line in the ovariole. When the oocyte reaches about half the size of the mature egg, yolk granules begin to appear in its cytoplasm. Follicular epithelium disappears in the last instars and the oocytes are covered with chorion. Prior to subimaginal ecdysis the eggs descend into the oviduct which is transformed into a sac-like organ. The germarium and nondeveloped oocytes degenerate. The ovariole of *Cloeon dipterum* usually contains only one egg, and the egg does not descend into the oviduct. This modification is due to ovoviviparity.

DISCUSSION

S. CIANCIARA: Could you observe any moment of the rapid growth or develop of the gonads in the development of *Cloeon dipterum*?

T. SOLDÁN: Yes, this moment can be observed more or less in the middle of the developmental period of these larvae.

U. HUMPECH: Did you observe the number of chromosomes of your species?

T. SOLDÁN: When using the method of staining by haematoxyline, the chromosomes sometimes can be visible only in a small number of cells, but this method is not suitable for counting of chromosomes. For counting of them another more precise and specific method is recommended (lactate-acetate-orcein).

U. HUMPEŠCH: How the spermatogenesis and oogenesis go on in parthenogenetic animals?

T. SOLDÁN: I have had no possibilities to examine parthenogenetic species of mayflies. Mostly parthenogenesis of mayflies is diploid. From unfertilised eggs only females are hatching. I think that oogenesis proceeds in the same way as in normal population but meiosis is suppressed.

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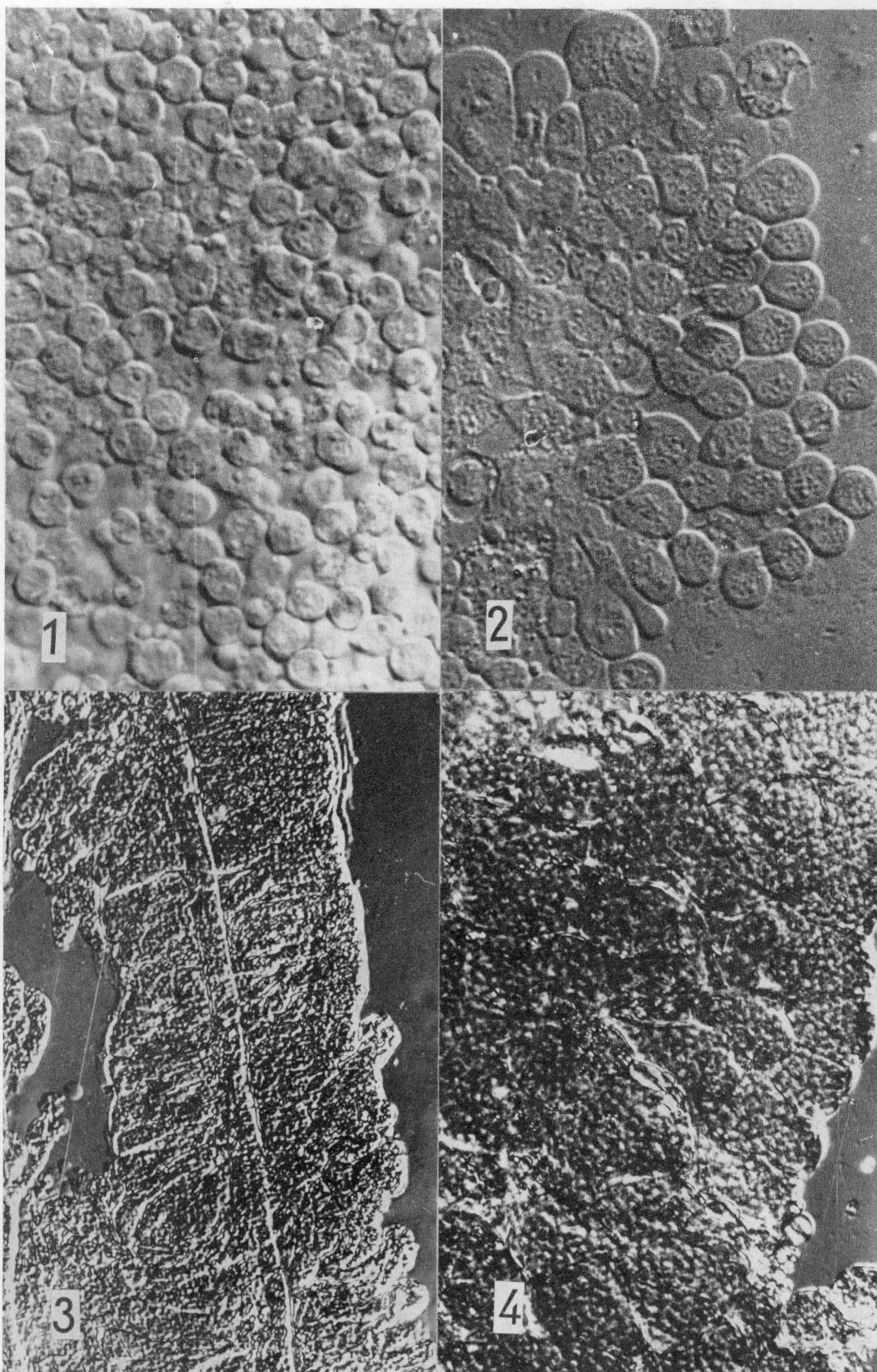


Plate I, Figs. 1-4: 1 — *C. dipterum*, larva, spermatogonia. Fast green, phase contrast. 2 — *C. dipterum*, larva, cyst with, spermatocytes. Fast green, phase contrast. 3 — *C. robusta*, larva, testis with vas deferens. Phase contrast. 4 — *C. robusta*, larva, detail of follicles filled by the cysts

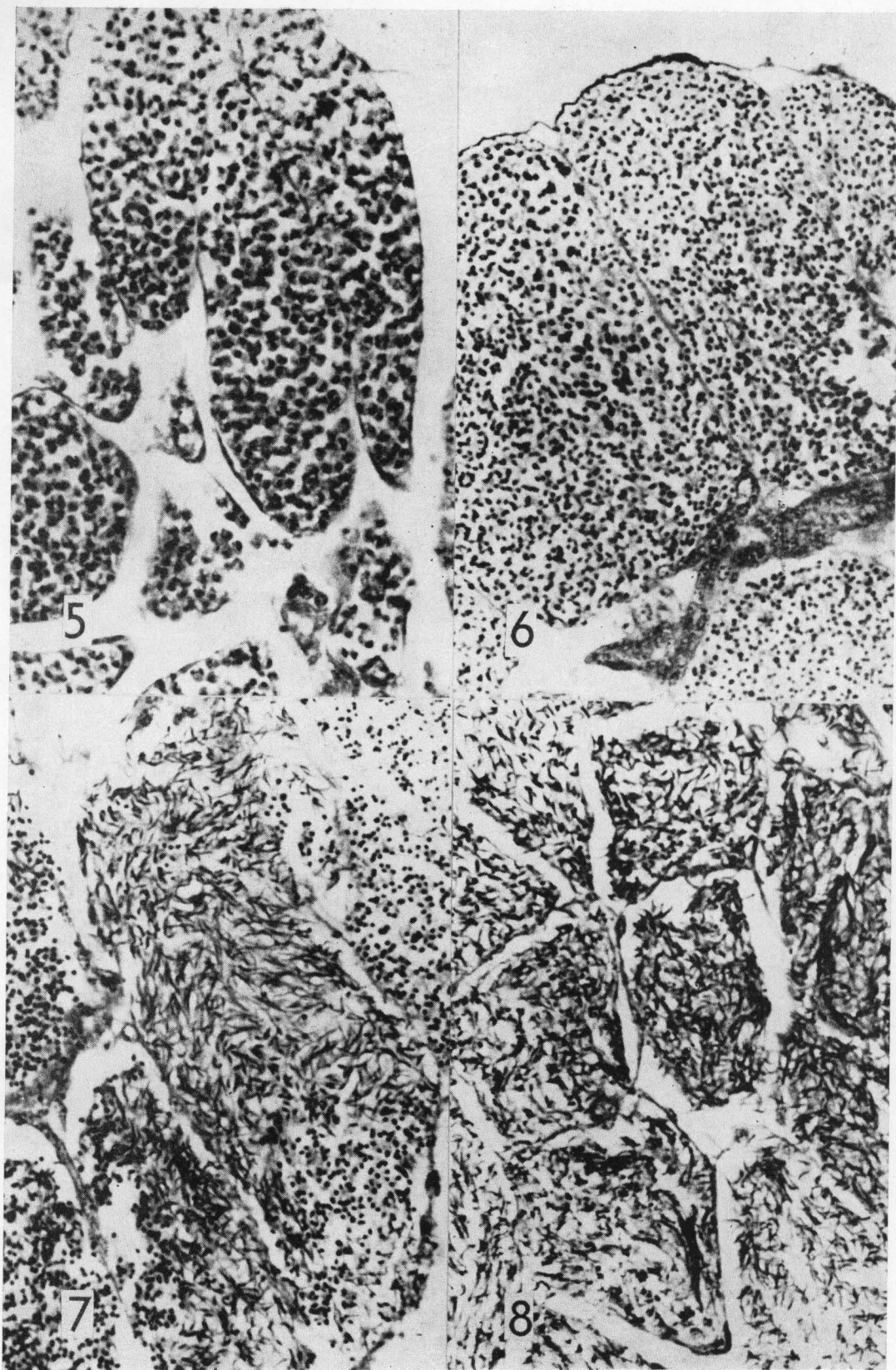


Plate II, Figs. 5-8: 5 — *E. torrentis*, larva, follicles with spermatocytes after desintegration of the cysts. BOUIN, HARRIS. 6 — *E. torrentis*, larva, follicles with spermatids and with the rest of spermatocytes. BOUIN, HARRIS. 7 — *E. torrentis*, larva, follicles with spermatids, praesperms and sperms. BOUIN, HARRIS. 8 — *E. torrentis*, larva before moulting, follicles with praesperms and sperms

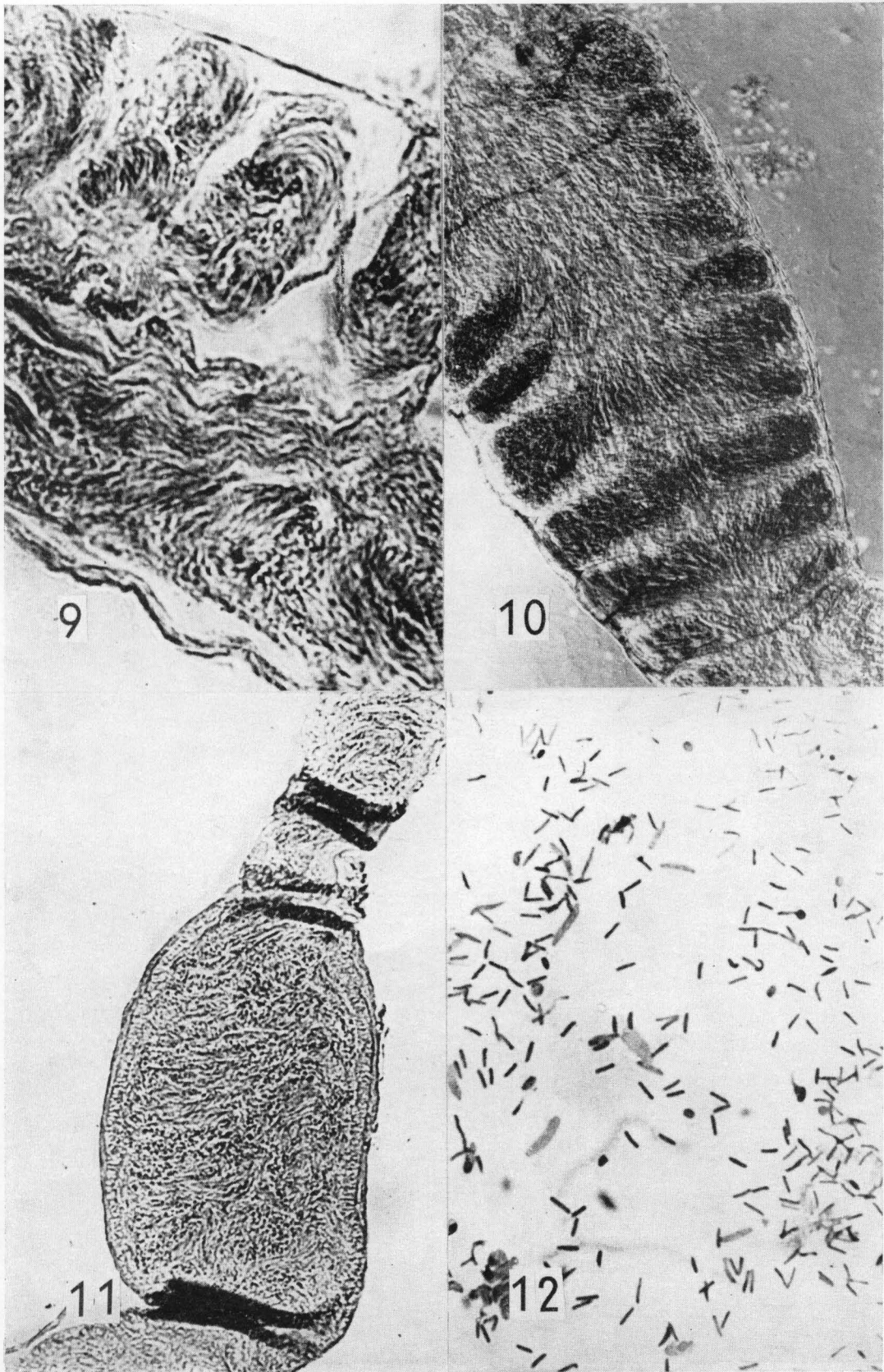


Plate III, Figs. 9-12: 9 — *E. danica*, larva before moulting, follicles and vas deferens filled by mature sperms. CARNOY, HARRIS. 10 — *E. torrentis*, imago, seminal vesicle. Phase contrast. 11 — *P. submarginata*, imago, seminal vesicle, CARNOY, HARRIS. 12 — *E. danica*, imago, smear of sperms, PAPPENHEIM

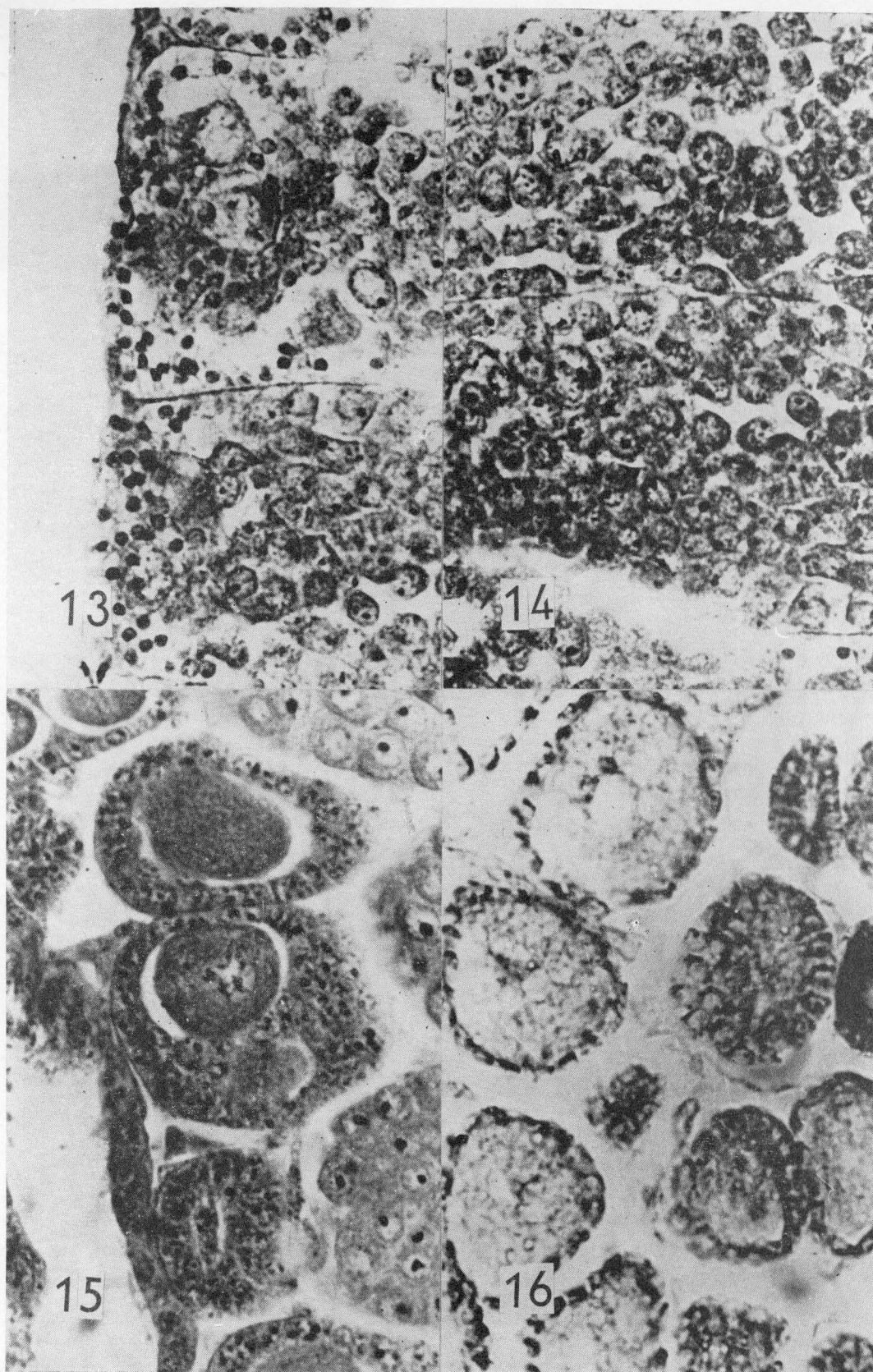


Plate IV, Figs. 13-16: 13 — *O. rhenana*, larva, ovariols with oogonia and oocytes. BOUIN, HARRIS. 14 — *O. rhenana*, oocytes, BOUIN, HARRIS. 15 — *E. danica*, larva, vesicula germinativa, CARNOY HARRIS. 16 — *P. submarginata*, larva, oocytes surrounded by the follicular cells. CARNOY, HARRIS.

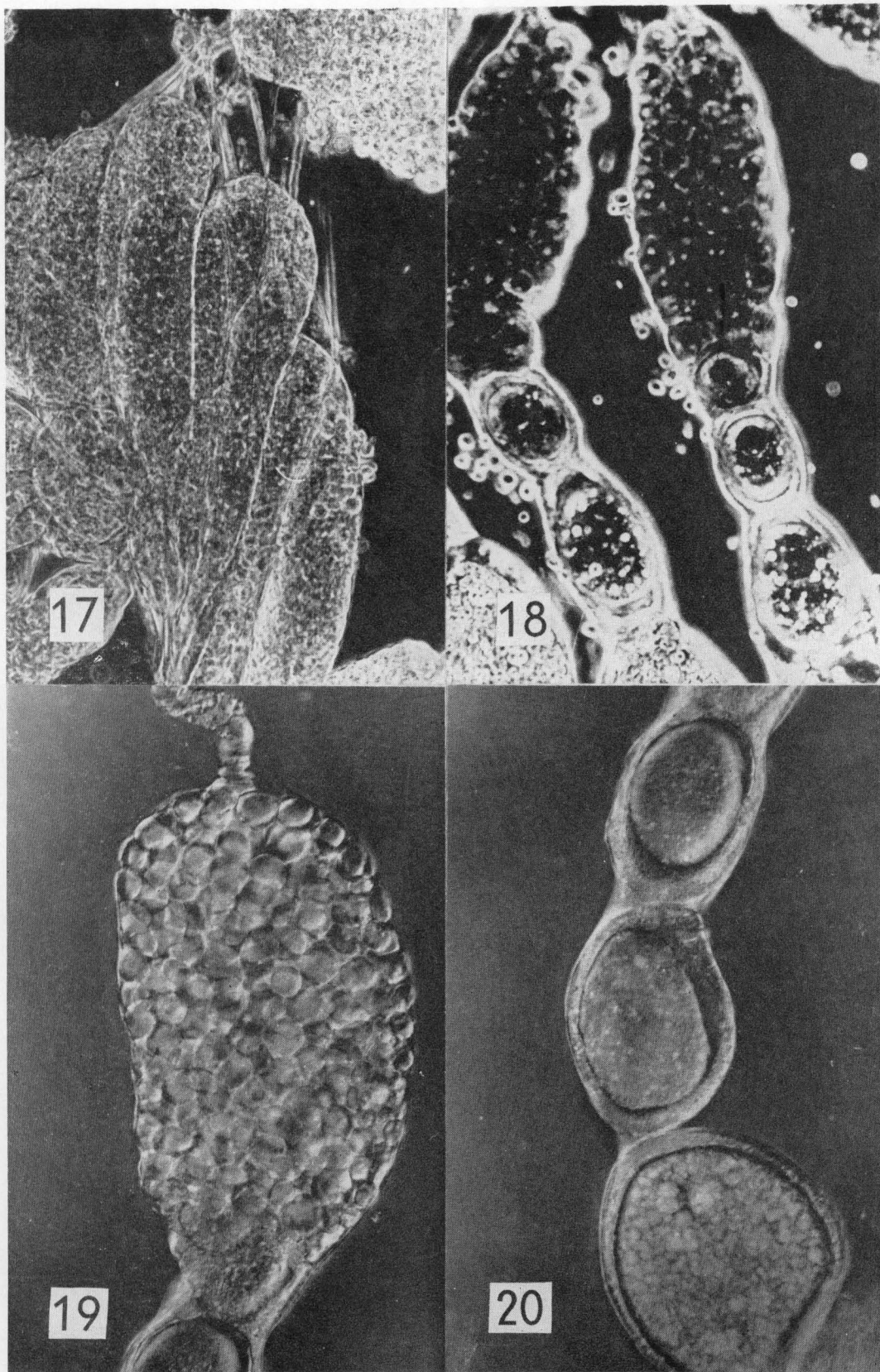


Plate V, Figs. 17-20: 17 — *C. robusta*, larva, ovariols with the terminal filaments. Anoptral contrast. 18 — *E. danica*, larva. Germarium and the oocytes increase in size. Anoptral contrast. 19 — *O. rhenana*, larva. Detail of germarium with the terminal filament. Interference contrast. 20 — *O. rhenana*, larva, oocytes aligned at the basis of ovarioli. Interference contrast

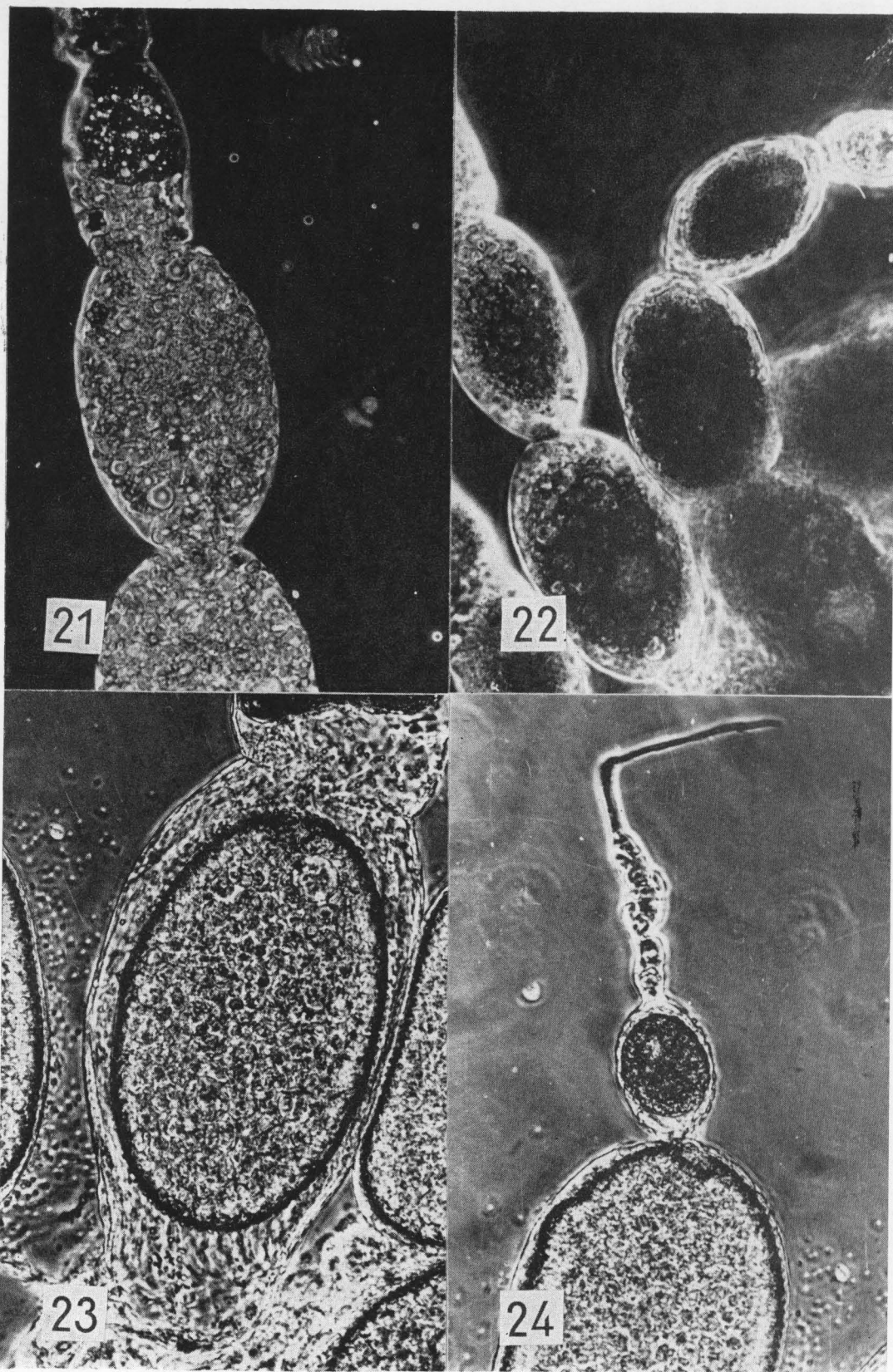


Plate VI, Figs. 21-24: 21 — *E. danica*, larva, vitellarium. Anoptral contrast. 22 — *P. submarginata*, larva, ovarioles with the first mature eggs. Anoptral contrast. 23 — *P. submarginata*, larva before moulting, mature egg after degeneration of follicular cells before descending into the oviduct. Phase contrast. 24 — *P. submarginata*, larva before moulting, degeneration of oocytes in germanium and younger oocytes in vitellarium. Phase contrast

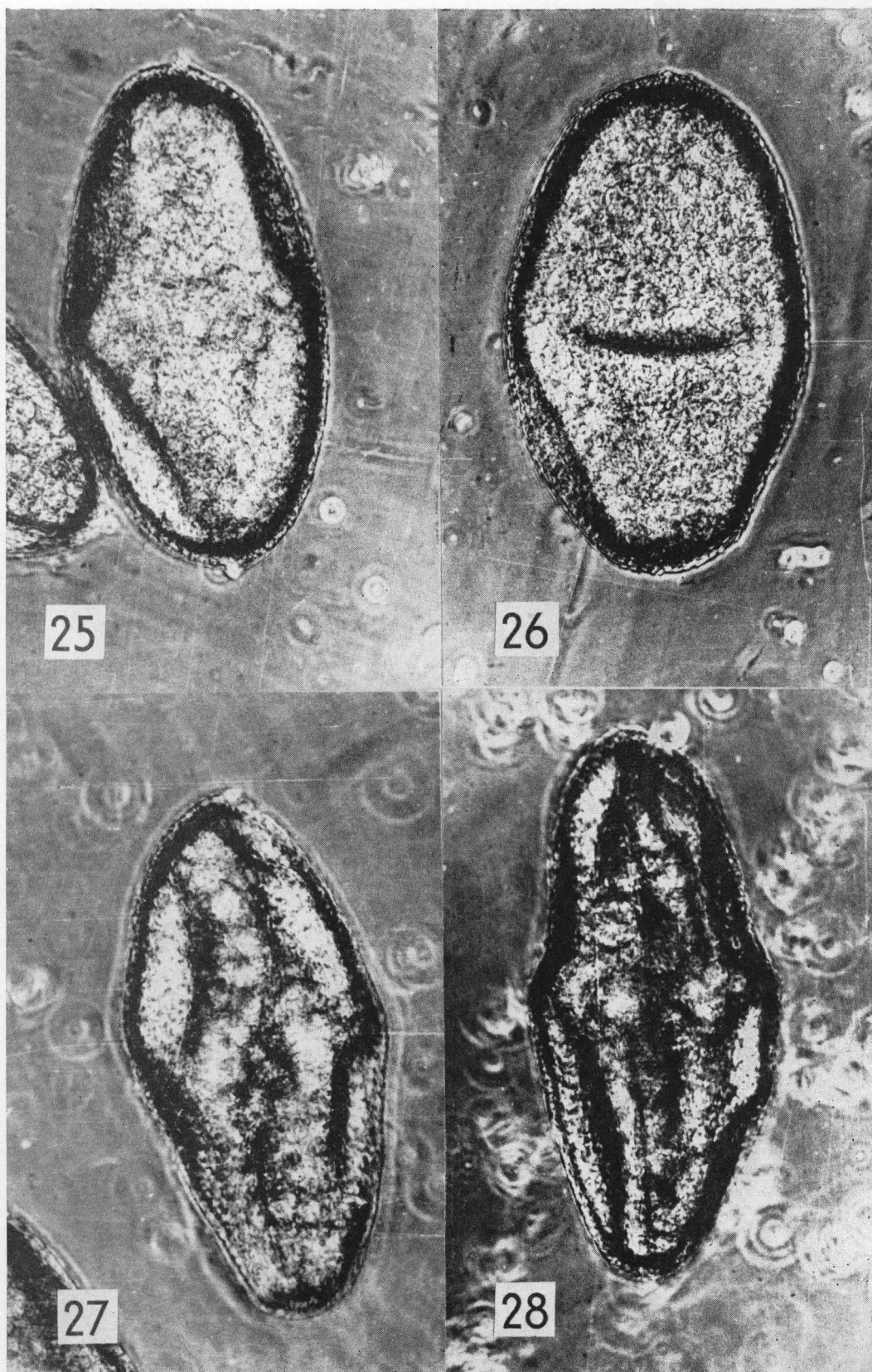


Plate VII, Figs. 25-28: *P. submarginata*, subimago. Various types of the changes of the shape of eggs in the oviducts during the subimaginal stage. Phase contrast