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THE EFFECT OF SYMBIOCLADIUS RHITHROGENAE (DIPTERA, CHIRONOMIDAE) ON THE DEVELOPMENT OF REPRODUCTIVE ORGANS OF ECDYONURUS LATERALIS (EPHEMEROPTERA, HEPTAGENIIDAE)

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Abstract. Results are given of studies on the effect of the ectoparasitic larva of *Symbiocladius rhithrogenae* on larval *Ecdyonurus lateralis*. In younger and older female larvae attacked by *S. rhithrogenae*, the division of oogonia and oocytes was discontinued, their nuclei became pycnotic, ovarioles and oviducts disintegrated and the larvae became sterile. A sterilisation due to the disintegration of the testis was observed in half-grown male larvae only, while in older male larvae, the presence of the parasite caused merely a merging of several testicular follicles and a marked enlargement of cells of the peritoneal epithelium. It had no influence on the spermatogenesis. Changes both in male and female larvae appeared at as late a time, at which the parasite was in its 3rd instar or pupal stage.

Larvae of *Symbiocladius (Phenocladius) rhithrogenae* Zavřel, 1924 have been shown to be ectoparasitic in mayfly larvae of the genera *Ecdyonurus* and *Rhithrogena*. They attack the larvae of their host at the time between approximately the 10th to 15th instar, never before or after this period. Larvae of the parasite attach themselves firmly to sites below the wing sheaths of their host, feed on its haemolymph and cause a discontinuation of the moulting process. Codreanu (1935, 1939) maintained that the presence of the parasite was responsible for a tumor-like proliferation in the thorax of the host. After the time of pupation of the parasite, the majority of older infected host larvae continue in their moults to the subimaginal or imaginal stage, while younger infected larvae die. Although a considerable knowledge is available of the life cycle of the parasite and its effect on larvae of its host, its influence on the development of the gonads of the host has not been evaluated so far with histological methods in spite of an evident influence of the parasite on the fertility of the adult mayfly whose larva had been infected. We have undertaken this study to determine the effect of the larval *Symbiocladius rhithrogenae* on the development of reproductive organs of the larval *Ecdyonurus lateralis* (Curtis, 1834) attacked by this parasite.

MATERIALS AND METHODS

Both infested and control larvae were collected in two localities of Central Bohemia (a brook at Barochov near Benešov; Zlatý potok, Davle-Libřice). Our material was collected in the summer months, at the time of development of both generations of the parasite (Codreanu 1934). Larvae were kept to the adult stage in a vessel with aerated water at a temperature of 15 ± 1°C. The age of the larvae was determined with Lehmkuhl's (1970) classification. Gonads and whole individuals were fixed with Bouin's fluid for 3—4 days for examination with histological methods. Sections (thickness 4—6 μm) were stained with Mayer's haematoxylin eosin, smears and squashes with Pappenheim's modified method. Of a total of 105 infested specimens and 57 controls, 42 were treated with histological methods.

RESULTS

THE DEVELOPMENT OF THE TESTIS IN THE CONTROLS

The testes of larvae are located laterally along the digestive system in abdominal segments I – VI, in older larvae, they reach the metathorax. The testis consists of a large number of testicular follicles enveloping the gonoduct from above and the sides. The follicles are equal in size, ovoid, joined directly to the gonoduct. The seminal vesicle is formed by a moderate enlargement of the gonoduct in segments VII – IX. The gonoduct consists of an outer muscular layer and an inner epithelial layer. In half-grown larvae, the follicles are packed with cysts containing spermatocytes – a layer of cysts with spermatocytes I distally, one with spermatocytes II proximally; a very narrow layer of spermatogonia (germarium) might be present in a few individuals. In older larvae, spermatids start to appear in proximally located cysts. They are overlaid by a wide layer of cysts with spermatocytes II, while a very narrow apical layer of cysts with spermatocytes I might persist in a small number of individuals. The germarium has disappeared completely from these larvae. The origin of spermatids is concomitant with the disintegration of the cysts. All spermatocytes change gradually into spermatids and cysts with spermatocytes I disappear from the follicles of larvae which are several instars before their moult to the subimaginal stage, while cysts with spermatocytes II form a narrow, apical layer. The first presperms appear in the last 2 – 3 instars preceding the moult to the subimaginal stage, follicular remnants are filled with spermatids, spermatocytes are almost absent. In the last instar, mature sperms descend to the gonoduct and the follicles disintegrate. Close to the subimaginal stage, almost all germ cells have changed into mature sperms, spermatids and presperms are scarce. Sperms concentrate in the greatly enlarged seminal vesicle, and the testis is visible only in abdominal segments III—IV. The seminal vesicle continues to widen during the subimaginal stage and all that remains visible of the testis is a moderately enlarged gonoduct and follicular remnants in abdominal segments V and VI. No further changes occur in the adult, all germ cells have attained the sperm stage.

THE DEVELOPMENT OF THE TESTIS IN INFECTED LARVAE

Half-grown larvae infected with 1st and 2nd instar larvae of the parasite were not different from the controls. Differences were found in larvae infected with 3rd instar larvae of the parasite evidencing themselves in a discontinuation of the division both of spermatogonia and spermatocytes (meiosis). The germarium was visible in all larvae. Cysts and the structure of follicles were unchanged. Changes in the testis were considerable in larvae infected with 4th instar larvae of the parasite. Although the testicles were still intact, cysts containing both spermatocytes I and II disintegrated. The testicles were packed with a conglobation of cells several with pycnotic nuclei which made it impossible to identify their origin. Pycnotic spermatocytes, spermatogonia and remants of cyst cells were present in the cellular conglobation. Both the gonoduct and the anlagen of the seminal vesicle still retained their structure. A total disintegration of the testis was observed in surviving larvae abandoned by the parasite's pupa. Individual testicles were joined one to the other and difficult to distinguish, the gonoduct had lost its structure and was merged with the joined testicles. The inside of the testicles of the testis was occupied by conglobations of pycnotic cells and pycnotic cells started to appear among these cellular conglobations (Plate I, Fig. 4). The testis was changed into an elongate, structureless formation occupying abdominal segments III – VI. While older larvae infested with the first and second instar of the parasite's larvae were not different from the controls, several testicular follicles (2–3 at the utmost) were joined into a single structure abutting the gonoduct (Plate II, Fig. 1) in older larvae infested with 3rd instar larvae of the parasite. However, the spermatogenesis was undisturbed, follicles were filled with spermatids and remnants of spermatocytes (Plate II, Fig. 1) either outside or still inside the cysts. Cells of an unusually large size organized in groups among testicular follicles (Plate I, Figs. 1, 2) were found in larvae infected with attached 4th instar larvae or pupae of the parasite. These greatly enlarged cells belonged to the peritoneal epithelium forming the wall of the follicles. Presperms started to appear in these larvae (Plate II, Fig. 2). Both the testicles of which several might have joined, and the gonoduct were well visible. The spermateleosis continued at a considerable rate in hosts which the pupa of the host had left. The follicles were filled with sperms and praesperms, the number of spermatids was reduced to a minimum, sperms were present already in the gonoduct (Plate II, Fig. 3). However, in comparison with the controls, the total disintegration of the follicles in association with the descent of sperms into the seminal vesicles proceeded at a slower rate, the testis was still clearly visible even in anterior abdominal segments and in the metathorax at a time close to the subimaginal stage. Therefore, follicles continued to disintegrate during the subimaginal stage although most of the germ cells had changed into adult sperms. The testis remained visible even in the first abdominal segments. We concluded from these fact that sperms continued to descend even during the adult stage. Morphologically, the sperms were conform to those of the controls (Plate II, Fig. 4).

THE DEVELOPMENT OF OVARIES IN THE CONTROLS

Ovaries of younger larvae were located along the digestive tube, in the metathorax and in abdominal segments I–VI, in older larvae they reached the mesothorax. The ovary consisted of a large number of ovarioles enveloping the oviduct from above and the sides. The oviduct was composed of an outer muscular layer and an inner epithelial layer. Ovarioles of half-grown larvae were formed by the germarium only, the vitellarium was still indistinct. Apart from a thin apical layer of oogonia, the germarium was filled with oocytes. Several slightly enlarged proximal oocytes were not yet surrounded by follicular cells. The apical layer of oogonia started to disappear from older larvae, the vitellarium was being formed. Proximal oocytes increased greatly in size and were surrounded by follicular cells producing a one-layered follicular epithelium. The vitellogenesis started rather late, i.e., at the time of formation of 3–4 egg chambers in the largest egg chamber, several instars before the subimaginal stage. At that time, eggs started to descend into the saclike enlarged oviduct. In the last instar preceding the subadult stage, almost all eggs were already in the greatly enlarged oviduct which occupied the entire space available in the abdomen and the thorax. Germaria of ovarioles and undeveloped oocytes in the last egg chambers were resorbed. No other substantial changes were observed in the subimago; mature eggs descended to a still unenlarged oviduct in segment VI.

THE DEVELOPMENT OF OVARIES IN INFECTED INDIVIDUALS

Similar to the male larva, the half-grown female larva infected with attached, lst- and 2nd instar larvae of the parasite, was not different from the control. However, 3rd-instar larvae of the parasite arrested the growth of oocytes and the division both of oocytes and oogonia. In larvae with anlagen of the first egg chambers, an infection with 3rd-instar larvae interrupted the formation of the follicular epithelium. Individual ovarioles and the oviduct were still visible, the incidence of pycnotic oocytes was solitary

(Plate III, Fig. 1). In half-grown larvae infected with attached, 4th-instar larvae of the parasite or its pupae, the ovarioles contained a large number of pycnotic cells, mostly oocytes or follicular cells (Plate III, Fig. 2), the germarium and the originating vitellarium could not be distinguished in the ovarioles, several ovarioles or their germania merged. In larvae surviving the departure of the parasite's pupa, we observed a complete disintegration of ovarioles which moreover joined with the oviduct into a homogeneous tissue consisting of cells with pycnotic nuclei (Plate III, Fig. 3). If these larvae possessed anlagen of the first egg chambers, the follicular epithelium around the pycnotic oocyte disintegrated (Plate III, Fig. 4). The oviduct persisted in the 6th abdominal segment, but the ovary was not extended into the thorax. Differences between controls and infected larvae appeared very late, i.e., at the 3rd-instar stage of the larval parasite. In larvae possessing only anlagen of the egg chambers, the disintegration of ovarioles was similar to that recorded for half-grown larvae (Plate IV, Fig. 1). In larvae with developed egg chambers, first minute vacuoles appeared in the ooplasm, then the karyolemma started to disintegrate, and the nucleus of the growing oocyte to dissolve (Plate IV, Fig. 2). An enlargement of vacuoles in the ooplasm and a complete disappearance of the nucleus were observed in larvae infected with attached 4th-instar larvae of the parasite or its pupae. The follicular epithelium of egg chambers was damaged in these larvae. A proliferation of the follicular epithelium was not observed, but the rate of growth of the individual cells was not uniform (Plate IV, Figs. 3, 4). In larvae with a starting vitellogenesis, the presence of the parasite arrested the deposition of yolk granules in the ooplasm, and was responsible for a further enlargement of vacuoles in it (Plate IV, Fig. 4). However, neither ovarioles nor the oviduct disintegrated. No further changes were recorded either for the subimago or the imago, because none of the oocytes matured and chorion secretion did not start. The ovaries of the adult were not extended into the thorax and resembled ovaries of older larvae.

DISCUSSION

The results obtained in the present study concerned with the influence of the parasite on the development of the reproductive organs of *Ecdyonurus lateralis* were in several points conform to those obtained by Codreanu (1939) in his detailed study on infected larvae of the genera *Rhithrogena* and *Ecdyonurus*. By contrast to this author, we did not observe such marked changes in the size both of male and female reproductive organs of the larvae, but in their histological structure. In comparison with the controls, the spermatogenesis of older male larvae was neither disturbed nor remarkably delayed, but there was a slight retardation in the spermateleosis and the descent of sperms to the seminal vesicles, terminating in these individuals in the subimaginal or even the imaginal stage.

In addition to a delay in the development of oocytes, and a discontinuation of the vitellogenesis, we observed a direct damage of the egg chambers in older females evidencing itself in a vacuolization of the ooplasm and a dissolution of nuclei or growing oocytes. According to Codreanu (1939), the destruction of the ovaries started as late as in last instar larvae, and the vitellogenesis was discontinued, because neoplastic leukocytes needed a supply of nutrition, and as a result of the development of a tumor-like proliferation. Our larvae did not possess hermaphrodite gonads observed by Codreanu and Codreanu (1933), but it might well be that greatly enlarged cells present in the peritoneal epithelium of older male larvae were mistaken for growing oocytes. However, in no case could these cells have been germ cells, because their nuclei were different in structure and they lacked a follicular epithelium. In all other points, our results were conform to those obtained by Codreanu (1939) for larvae of *Rhithrogena semicolorata* (Curt.).

With regards to interactions between the parasite and the reproductive organs of the insect, it is quite exceptional that the male remains fertile and the female becomes completely sterile. Generally, the situation is reversed: a heavy damage of the male gonads results frequently in a 100 % mortality, while the female remains partly fertile at a very low mortality. Such cases have been recorded for mayfly species, e.g., for larvae of the genus *Cloeon* infected with microsporidians *Nosema baetis* Kudo, or larvae of the genus *Baetis* infected with parasitic worms of the family Mermitidae. The mortality of males was 100 %, several females retained partly their fertility. A total castration and a masculinizing of female larvae might occur in the genus *Baetis* (Degrange 1960, Arvy and Peters 1973). However, in both cases the parasites concerned were endoparasites. Apart from a total weakening of the host related with a loss of haemolymph, ectoparasitic larvae of *Symbiocladius rhithrogenae* can influence the development of internal organs of their host solely by means of biochemically active substances released evidently by salivary glands. It is almost certain that the parasite influences the development of the host and subsequently that of its reproductive organs by way of the humorally regulative system, but this hypothesis will have to be supported by detailed studies on relationships of neoplastic growth and the development of internal organs of the host. The damage of reproductive organs of mayfly larvae is similar to that caused by several classical chemosterilants and juvenoids with a sterilizing effect except for the fact that we observed neither a proliferation of the follicular epithelium nor a partial renewal of the female fertility.

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ВЛИЯНИЕ *SYMBIOCLADIUS RHITHROGENAE* (DIPTERA, CHIRONOMIDAE) НА РАЗВИТИЕ ПОЛОВЫХ ОРГАНОВ *ECDYONURUS LATERALIS* (EPHEMEROPTERA, HEPTAGENIIDAE)

Т. Солдан

Резюме. Изучали влияние эктопаразитической личинки *Symbiocladius rhithrogenae* на личинки *Ecdyonurus lateralis*. У личинок самок *E. lateralis* среднего возраста и старших пораженных *S. rhithrogenae* остановилось деление оогоний и ооцитов, их ядра становились пикнотическими, овариолы и яйцеводы разрушились и личинки стали стерильными. Стерилизация, причиненная распадом семенника, наблюдалась только у личинок самцов среднего возраста. У старших личинок происходило только сливание некоторых тестикулярных фолликулов и значительное увеличение клеток перитонеального эпителия, тогда как сперматогенез не нарушился. Изменения в личинках самцов и самок проявляются только в то время, когда паразит в стадии личинки III или в стадии куколки.

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EXCYSTATION OF SCOLECES OF CESTODES OF THE FAMILY HYMENOLEPIDIDAE FROM THE CYSTS IN VITRO

A method of releasing scoleces from the cysts was used for detailed studies on the morphology of cestode cysticercoids belonging to the family Hymenolepididae Fuhrmann, 1907: *Dicranotaenia coronula* (Dujardin, 1845), *Diorchis inflata* (Rudophi, 1819), *D. nyrocae* Yamaguti, 1935, *Fimbriaria fasciolaris* (Pallas, 1781), *Microsomacanthus compressa* (Linton, 1782), *M. paracompressa* (Czapliński, 1956), *M. paramicrosoma* (Gasowska, 1931), *Sobolevicanthus gracilis* (Zeder, 1803) and *S. octacantha* (Krabbe, 1869) whose larval stages develop in planktonic crustaceans of the classes Copepoda and Ostracoda.

Gastric juices of a duck (*Anas platyrhynchos domestica* Linné) starving for 12 hours and a 0.1 % solution of synthetic trypsin by Hoffman (Trans. Am. Fisheries Soc. 88: 96—99, 1959) were used for excystation. All experiments were conducted at the temperature of 39 °C. At first, isolated cysticercoids were exposed to fresh, undiluted gastric juices, and after 50—60 minutes they were transferred to the solution of trypsin. In the course of 10—15 minutes ruptures occurred at sites of invagination; and at first the scolex, later the neck appeared separated or occasionally connected with the cyst by a narrow strip of tissue. At this instant, the solution of trypsin had to be diluted by distilled water (1 : 1) lest partial digestion of the scolex should occur.

A converse method, i.e. an application of a 0.1 % solution of trypsin followed by that of gastric juices of a duck as used by Ždárská (Čs. parasitol. (Praha) 11: 343—345, 1964) in excystation of the metacercariae of echinostomous trematodes was not effective. Diluted gastric juices as well as lower concentrations of synthetic trypsin were also without effect. Some experiments have been carried out with slightly injured bodies of crustaceans infected with cysticercoids. In these cases, exposition of the cysticercoids to gastric juices had to be prolonged to 80—90 minutes. Other methodical procedures which were used in excystation of scoleces of different cestode species by Rothman (Exp. Parasitol. 8: 336—364, 1959), Sawada (Exp. Parasitol. 8: 325—335, 1959) and Smyth (Commonwealth Bureau Helminthol. 34: 3—38, 1963) gave no positive results in our experiments.

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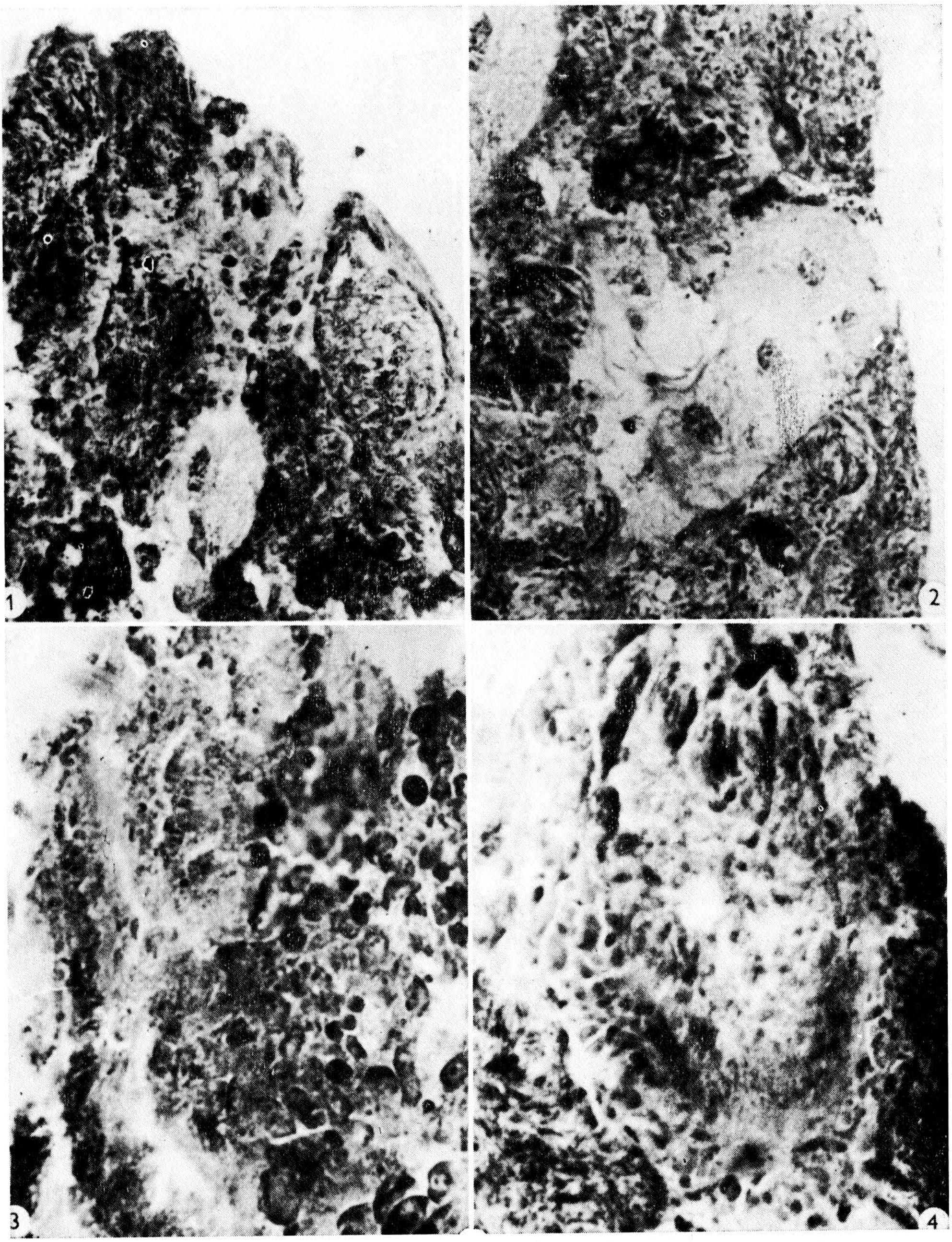

Fig. 1. Section through testis of older larva. Testicles filled with enlarged peritoneal cells. **Fig. 2.** idem, detail of enlarged peritoneal cells. **Fig. 3.** Section through testis of half-grown larva; testicles disintegrated, remnants of pycnotic spermatocytes. **Fig. 4.** section through testis of half-grown larva after the disintegration of the testicle with remnants of necrotic tissue and a vacuole. 1—4 HE, ×200.

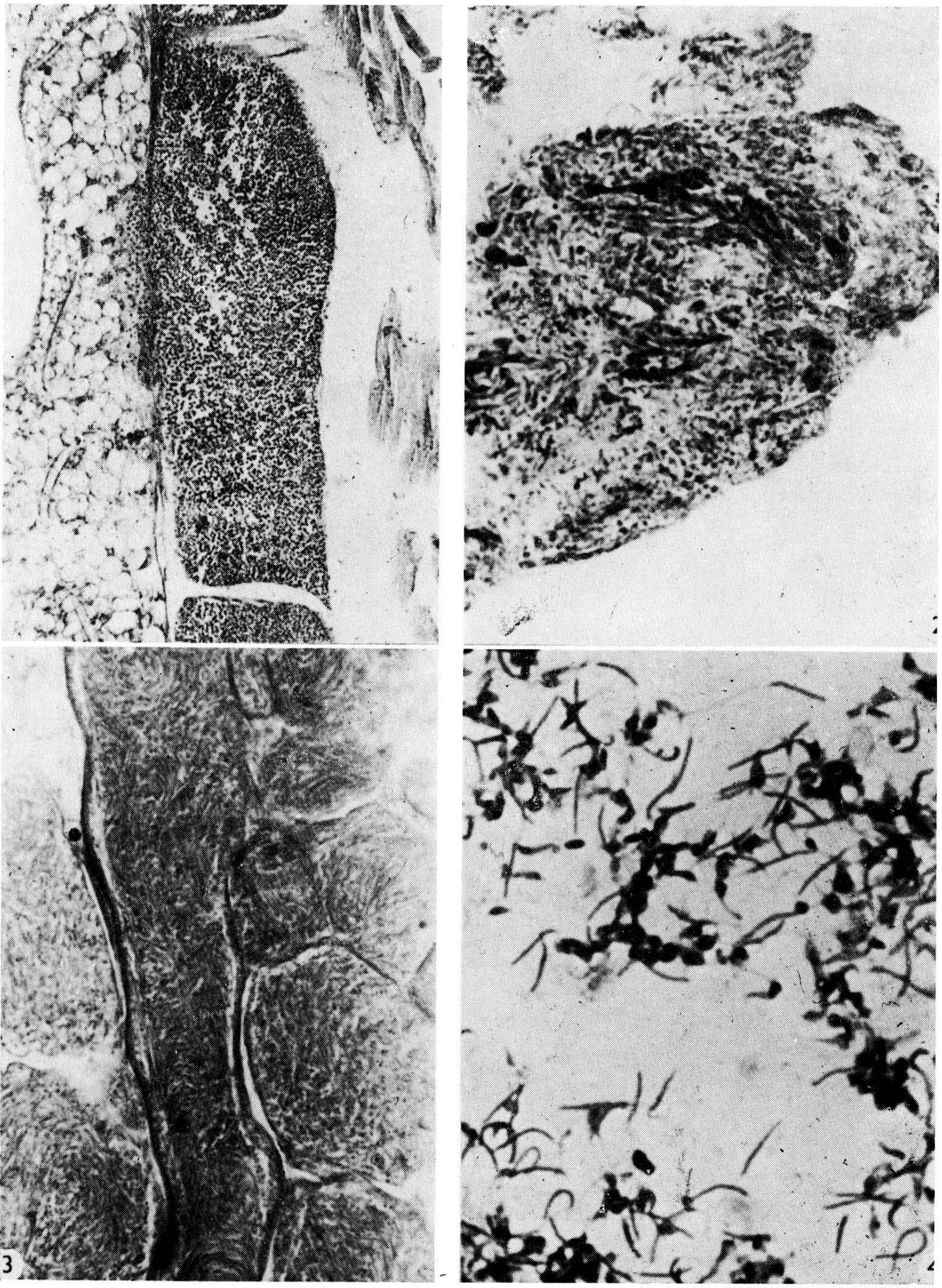

Fig. 1. older larva, section through joined testicles filled with spermatids. **Fig. 2.** older larva, section through testicle with a starting spermateleosis. **Fig. 3.** mature larva (after the detachment of the parasite's pupa), section through the gonoduct and testicles with sperms and remnants of spermatids. **Fig. 4.** adult (from an infected larva), smears of sperms. 1—3 HE ×200, 4 Pappenheim ×800.

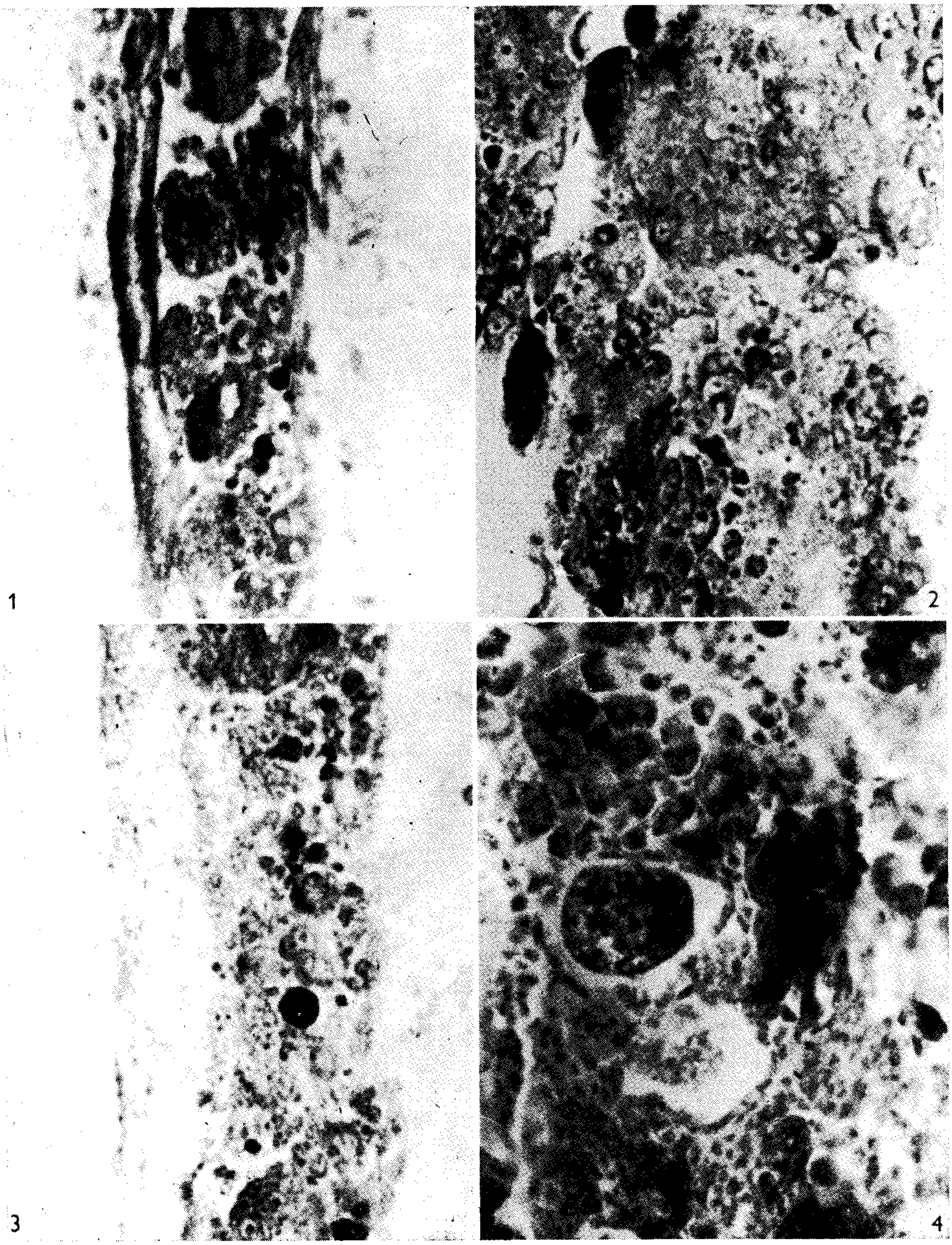

Fig. 1. Half-grown larva, section through ovary with pycnotic oocytes. **Fig. 2.** idem, detail of pycnotizing oocytes in germarium. **Fig. 3.** half-grown larva, section through a disintegrated previtellarium and the first egg chamber. L-4 HE ×200.

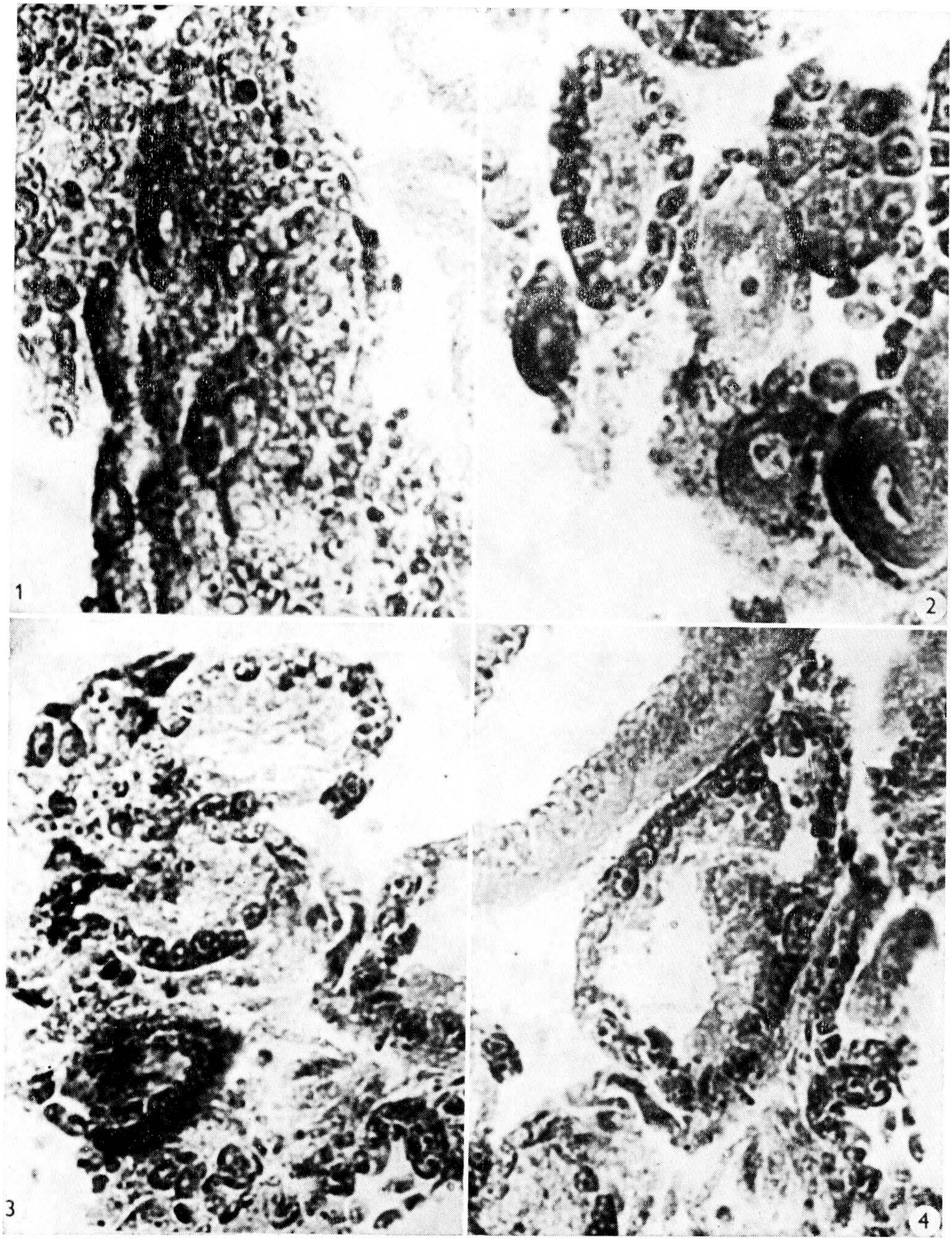

Fig. 1. older larva, section through damaged egg chambers (disintegration of the nucleus, degeneration of the follicular epithelium, vacuolization of the cytoplasm). 1—4 HE, ×200.