〔寄生虫学雑誌　第12巻　第6号 497-501頁　1963〕

STUDY ON *ONCOMELANIA NOSOPHORA* INFECTED WITH *SCHISTOSOMA JAPONICUM*

— Gametogenesis —

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(Received for publication, September 23, 1963)

The studies on the snail-dwelling larvae of *Schistosoma japonicum* were already made by many investigators (Ota, 1957; Nagasaki, 1960; and others). Ota (1957) postulated that the presence of larval trematodes appears to injure the tissue of the snail host. On the other hand it was shown by Hashimoto (1959) that the growth of germ cell was related to the shell length of *Oncomelania nosophora*. Nagano (1963) emphasized that *Parafossarulus manchuricus*, an intermediate host of *Clonorchis sinensis*, was castrated and the life span of infected snails was shortened by infection of the flukes.

In spite of numerous studies, few have so far been made on the influence of schistosome infection upon the reproductive organ of molluscan intermediate host, *Oncomelania nosophora*. Yet the changes in gametogenesis of the host snail induced by the trematode infection are of considerable importance in connection with the information of population density of snails in nature.

The observations were carried out on morphological changes in gametogenesis of *O. nosophora* infected with *S. japonicum*.

Materials and Methods

Uninfected and infected *Oncomelania nosophora* of Kofu origin were maintained in the laboratory under a moist condition. For the preparation of infected snails, miracidia were collected from the feces of the dog previously infected with *S. japonicum* and allowed to hatch by the technique of Takahashi *et al.* (1961). The exposure of the snails to miracidia is essentially the same technique as that reported by Okamoto (1963).

For the microscopical observation the snails were fixed with Bouin's solution after 16 weeks of miracidial entry. The materials were sectioned longitudinally at 10 μ thickness and stained routinely with hematoxylin and eosin.

Results

Preliminary observations were carried out to examine whether the sex difference of host snails would affect the rate of infection; the proportion of snails in which the miracidial entry was established. Such examinations were made in both field (naturally infected snails) and laboratory (experimentally exposed snails). The data are summarized in Table 1.

Fourty-nine of 385 females and 24 of 179 males collected from the field in 1957 were cercarial positive. While in the laboratory examination, 48 of 90 females and 19 of 42 males had schistosome larvae. In both field and laboratory, the infection rate of female snails was almost the same as that of male snails since the Chi-square values are much smaller than 6.635 ($P=0.01$). This indicated no sex difference in the infection rate.

Oogenesis The oocytes at various stages of "growth period" were peripherally located in the ovary of the uninfected snail (Photo. 1). Occasionally, immature oocytes, in which the nuclei were slightly enlarged in size, were seen at high magnification. In immature stages the cytoplasm of oocytes was as much

Table 1. Comparison of schistosoma infection between male and female snails

	Sex	No. of uninfected snails	No. of infected snails	Total
Field	Male	155	24	179
	Female	336	49	385
	Total	491	73	564
		$\chi^2=0.047$		
Laboratory	Male	23	19	42
	Female	42	48	90
	Total	65	67	132
		$\chi^2=0.739$		

basophilic as that of young oocytes. Nucleus and cytoplasm of normal oocytes showed considerable changes in structure as they enlarged in size. The nuclei which always contain a large amount of dense material were observed in the immature oocytes. On the other hand the nuclei of mature oocytes, the so-called germinal vesicle, are very huge in size and contain one or two sperical nucleoli and chromatin which had peculiar appearance. While a number of fat-like droplets were observed as one of the intracellular inclusions of mature oocytes.

It appears that the oogenesis of infected snails is suppressed as compared with that of uninfected ones. In general, the oocytes observed in the infected snails were abnormal in shape and the nuclei (germinal vesicles) showed an indistinct structure (Photo. 2). The oocytes decrease in number and the ovary becomes vacant. The presence of such abnormal oocytes in almost empty ovaries suggests that a disturbance which was probably induced by the miracidial infection was occurred during the "growth period" and they probably lost their reproductive function.

Spermatogenesis The testes of uninfected snails are shown in Photos. 3 and 4. Different stages of spermatogenesis is recognizable in Photo. 4. Whereas such progressive stages in spermatogenesis could not be found in the testes of infected sanils. Using high magnification, it was observed that sperm which had already been produced in infected snails is microscopically normal in structure. The testes were remarkably shrunken in shape as compared with that of uninfected one (Photo. 5). The difference that was noted in the testes between infected and uninfected snails seems to give the impression that a disturbance observed in infected snails may be occurred in the process of meiosis but not in spermiogenesis, because of the presence of normal sperm in the testes of infected snails.

Discussion

Until recently a considerable amount of investigation has been performed on the ecological factors; temperature (Kawamoto, 1954), light (Kawamoto, 1954; Yasuraoka, 1955; Nakao *et al.*, 1958), humidity (Okabe *et al.*, 1956; Komiya *et al.*, 1959), soil (Okabe *et al.*, 1959; Komiya *et al.*, 1959), pH (Yasuraoka, 1959) and natural enemy (Iijima *et al.*, 1963), all which appear to influence the survival of the host snail.

The reasons why the author thought that these observations should be done are that few have so far been made on the influence of miracidial infection upon the snail behaviors and that there is no description in which the ecological distribution of snails was considered from a viewpoint of vector-parasite relationship itself.

The previous report (Okamoto, 1963) strongly seggested that the survival of infected snail was inferior to that of uninfected one; however there was no proof that they had reproductive capacity as well as that of uninfected one. The results obtained from preliminary examination indicated no sex difference in the infection rate. This fact might at least indirectly show that the study on the effect of trematode infection on the vector snails could be carried out from a viewpoint without respect to their sexes. Remarkable disturbance of gametogenesis was observed in the infected snail. This seems to be a rather general feature, since the same results have been found in other molluscan intermediate hosts, *Parafossarulus manchuricus* (Nagano, 1963)

and *Australorbis glabratus* (Okamoto, unpublished data). If the disturbance of gametogenesis by schistosomal infection was occurred naturally in the endemic area, one would expect that the population density of snails would be influenced significantly by the presence of miracidia.

Although this assumption described above cannot be safely emphasized, because of the absence of direct proof on the reproducibility of infected snail, the results obtained here would have been especially interesting for the consideration on the ecological feature of *Oncomelania nosophora*. Future studies will attempt to examine the effect of miracidial infection on the reproductive capacity and snail population density.

Summary

1. Oogenesis of infected snails was abnormal as compared with that of uninfected ones.

2. The testes of infected snails were remarkably shrunken in shape though normal sperm was present. In spite of the presence of normal sperm, meiosis could not be found in such infected male snails.

3. There is presumptive evidence that the gametogenesis had not undergone complete development in the infected snails.

Acknowledgement

The writer was aided in obtaining the necessary material by Dr. Toshihiko Iijima, the Yamanashi Prefectural Higiene Laboratory, Kofu. For his advice and encouragement as well as the care with which he read the manuscript, the writer is deeply indebted to Prof. Kazuo Mori, Showa Medical School.

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日本住血吸虫感染ミヤイリガイの研究
—偶配子形成—

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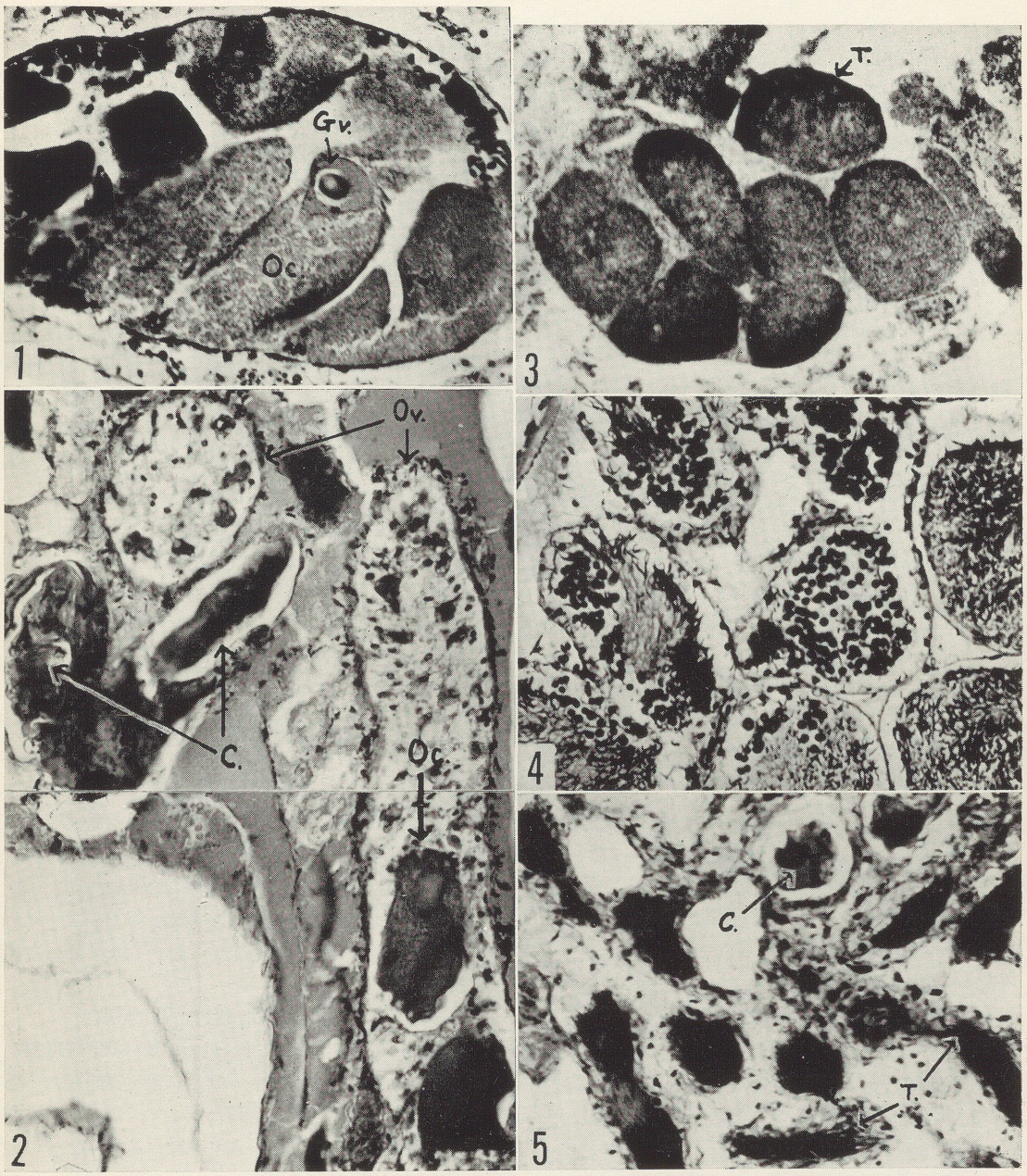

Explanation of Plate

1. Ovary of uninfected snail. Several oocytes (Oc.) are seen and some of them have germinal vesicle (Gv.) in which a large nucleolus exists. X 300.
2. Ovary of infected snail. Mature cercariae (C.) are seen and the ovary is almost vacant. Oocyte (Oc.) is irregular in shape and nucleolus is indistinct. X 300.
3. Testes of uninfected snail. X 100.
4. Spermatogenesis of uninfected snail. Different stages are recognizable. X 200.
5. Testes of infected snail. A cercaria (C.) is located near testes. The testes show an abnormal feature. X 300.