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Photomicroscopic Observation of Infective Cysticercoid and the Earlier Stages of *Hymenolepis nana* Isolated from the Mouse Intestine

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Introduction

The tissue stages of *Hymenolepis nane* developed in the beetle intermediate hosts or in *in vitro* culture have been thoroughly illustrated (Voge and Heyneman, 1957 ; Schiller, 1959 ; Seidel and Voge, 1975). However, there is a few observation of the tissue stages in the mouse intestine (Hunninen, 1935 ; Bailey, 1951), especially on the process of excystment (Caley, 1975).

In the present work we examined to see whether all the tissue stages in the mouse intestine could be easily collected by Ito's simple method (1977) and to observe the development of the infective cysticercoids isolated from the intestine.

Materials and Methods

Worm-free, 5±1 week old mice of dd strain (random-bred) were given 20,000 or more shell-free eggs each (day 0). On every day post egg inoculation (day 1, 2, 3, 4 or 5), a total of 10 to 15 mice was killed. The tissue stages were separated from the intestinal walls by Ito's method (1977) and observed under ×40, ×100 and ×200 magnifications.

The method for maintaining both the parasite and the host has been previously described (Ito and Yamamoto, 1977).

In this text, for example, 2-day worm means the worm separated from the intestine on day 2 (48±1 hr post egg inoculation).

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Observation and Discussion

Application of the simple method to all the tissue stages

A number of 2-day to 5-day worms other than 1-day worm (spherical body) were easily collectable throughout the experiments. The worms collected are illustrated in Figs. 1–21. The key to collect a number of worms is to repeat washing of the worms in the tissue debris with 0.9% NaCl solution, especially in collecting 2-day worms (Figs. 1–3). Thus the method was slightly modified; a step of stirring the filtrate in a glass beaker by magnetic stirrer for about 30 min at room temperature was inserted before washing on a round-bottomed dish (Ito, 1977).

One-day worms separated from the intestinal walls (Fig. 21) could not be collected without the tissue debris by the simple method.

Development of infective cysticercoids

Three-day worms were immature cysticercoids which had never accomplished the formation of adult hooks (Figs. 4–6). Four-day worms were similar to the stage V of tailed, beetle-derived cysticercoids (Voge and Heyneman, 1957) : the scolex of almost all the worms was always oriented anteriorly. Five-day worms (Figs. 10–20) were vigorously active and preparing for excystment : most of the worms had become adult form (Figs. 14–17) (the primordium of the future segments of the strobilate organism) and were wriggling about in the cyst walls until rupturing the walls (*c. f.* Caley, 1975).

When cyst walls of either 4-day worms

(Fig. 8) or 5-day worms (pre-adult form, Figs. 10–13) were ruptured mechanically, most of the worms could not completely take off their walls (Fig. 18a), and even if they could, the neck end of excysted worms was not closed (Fig. 9), whereas adult forms (Figs. 14–17) quickly took off the walls (Figs. 18b and 19) and the neck end was complete (Figs. 18b and 20). These observations may support Ito's work (1977) that the 5-day worm is more infective than the 4-day one, although the scolex has fully developed by day 4.

Massive collection of all the stages of *H. nana* in the mouse intestine seems to be very useful for analyzing the parasites' antigenic changes throughout their development in the immunized host (Ito and Yamamoto, 1977; Ito, *et al.* 1977). The stage that we could not collect without the tissue debris was the oncosphere which invaded the villi (1-day worm, Fig. 21). Thus other delicate method should be applied to prepare the oncospheres in the villi.

Summary

The simple method for collecting infective cysticercoids of *Hymenolepis nana* from the mouse intestine (Ito, 1977) was applied to collecting the earlier tissue stages. All the tissue stages other than 1-day worm (spherical body) could be easily collected. The process of excystment of the worms separated from the intestine and freed of the tissue debris was observed under ×40, ×100 and ×200 magnifications. Five-day worm (adult form) was defined to be infective cysticercoid, although the scolex of 4-day worm had fully developed.

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小形条虫のマウス小腸絨毛内発育幼虫の分離と脱嚢過程の観察

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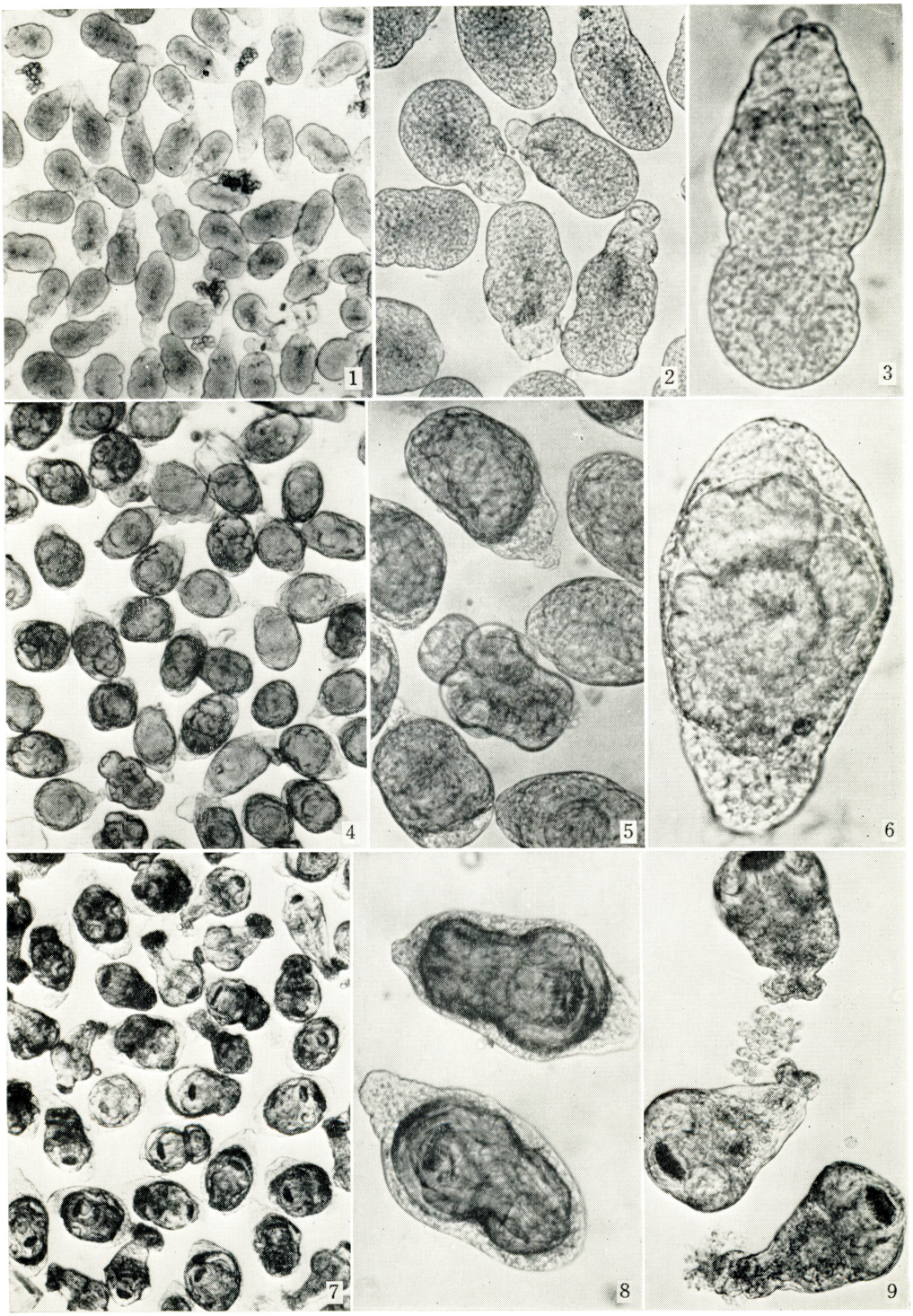
マウス小腸絨毛内発育幼虫の分離収集を伊藤の方法(1977)によつて試みた．虫卵投与後2日目以後の幼虫は簡単に大量に集められたが，1日目の幼虫は集められなかつた。

1日目から5日目までの分離幼虫を写真で示した．特に5日目の感染型擬嚢尾虫の脱嚢過程を詳しく観察した。

Explanation of Figures

Fig. 1–9　Tissue stages of *Hymenolepis nana* prepared from the mouse intestine. 1–3 : Two-day worms (1 : ×40, 2 : ×100, 3 : ×200). 4–6 : Three-day worms (4 : ×40, 5 : ×100, 6 : ×200). 7–9 : Four-day worms (7 : ×40, 8 and 9 : ×100). 9 : Excysted 4-day worms.

Fig. 10–21　Tissue stages of *H. nana* prepared from the mouse intestine. 10–20 : Five-day worms, ×100. 10–13 : Pre-adult form in the cyst wall. 14–17 : Adult form in the cyst wall. 18a : Pre-adult form under excystment. 19 : Adult form under excystment. 18b and 20 : Excysted worm (*c. f.* Fig. 9). 21 : One-day worm. ×100.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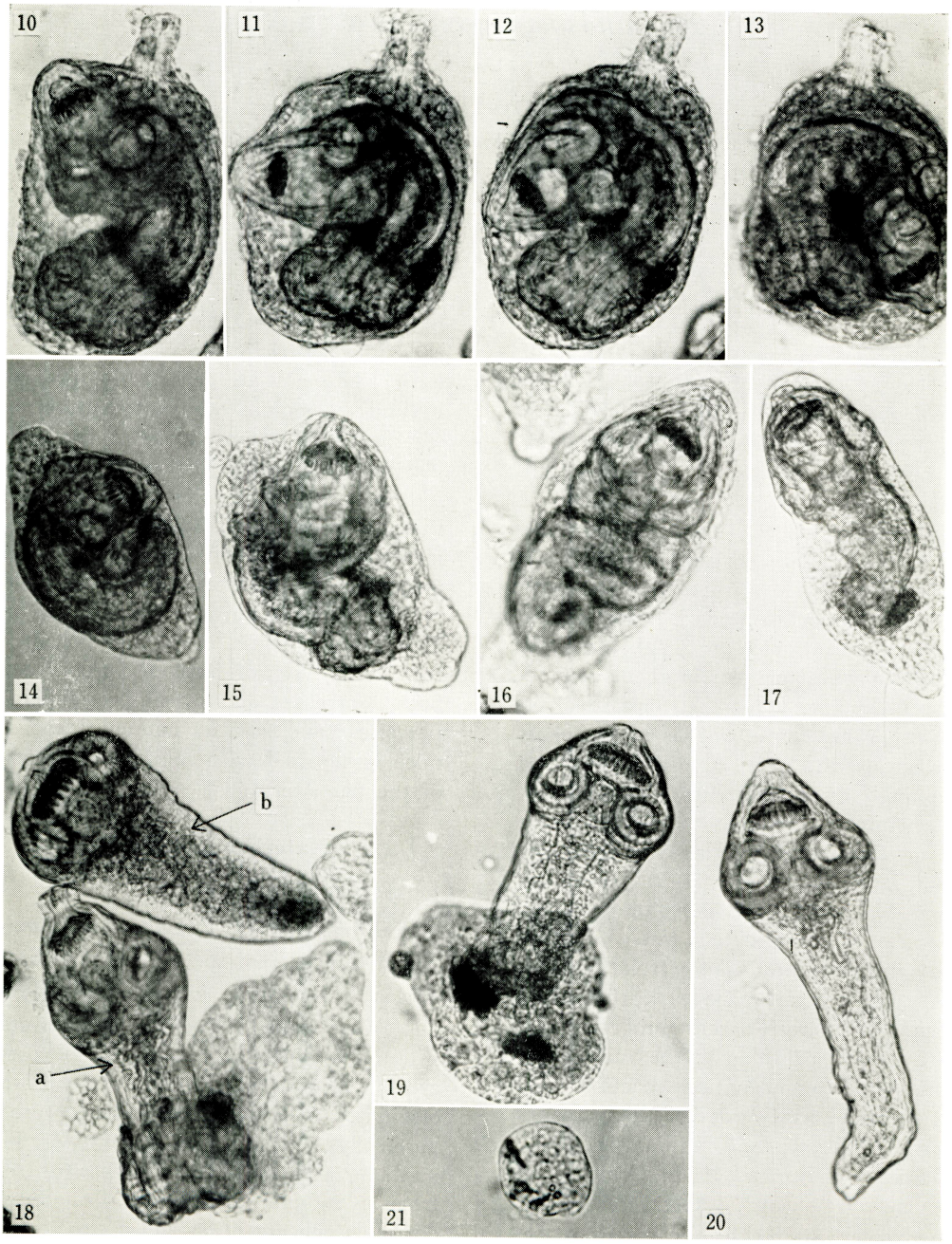
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