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Intestinal Pathology Associated with Primary and Secondary Infections of *Hymenolepis nana* in Mice

TAKASHI MIYAZATO, TADAAKI FURUKAWA
AND TOMOYOSHI INOUE
Department of Parasitology, Kinki University School of Medicine, Osaka, Japan

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Introduction

The marked protective immunity against *Hymenolepis nana* infection in mice has been well documented (Gemmell, 1976), but the mechanism of the worm expulsion from previously infected mice is unknown. Bailey (1951), dealing with the histological observations on the host-tissue reactions to primary and secondary infections with *H. nana* in mice, proposed that the great majority of the oncospheres are unable to penetrate the intestinal mucosa of the previously infected mice, and the few larvae which are able to gain access to the lamina propria of the intestinal villi are killed by the accelerated host-tissue reactions. However, his observations on the intestinal pathology have based on a small number of animals and the cellular reactions of the intestine in response to secondary infection was not fully investigated. In addition, the chronological order observation made at about 24-hour intervals after infection (Bailey, 1951) appears to be insufficient, since (i) the protective action of previously infected mice is considered to be directed against the oncosphere before or shortly after penetration of the intestinal mucosa of infected mice (Ito and Yamamoto, 1976), and (ii) the penetration of *H. nana* oncospheres into the intestinal mucosa is known to be a fairly rapid process (Miyazato *et al.*, 1977). The lack of detailed observations on the histopathology due to *H. nana* infection in mice prompted the present work, and we paid a special attention to the fate of oncospheres derived from the secondary infection at the early stage of infection.

Materials and Methods

The maintenance of *H. nana* life cycle in ddY mice, collection of mature eggs, removal of egg-shells before infection and the method of oral infection have been described previously (Furukawa, 1974).

Sixty ddY mice were weaned at 3 weeks of age and when 8 weeks old, they were randomly divided into three groups. Twenty eight mice were infected orally with 10,000 shell-removed eggs of *H. nana* each and two pairs of infected mice were killed 4, 8, 16, 24, 48, 72 and 96 hours after infection. These mice given only primary infection were referred to as nonimmunized mice. Another 28 mice were initially infected orally with 10,000 eggs. Ten days later they were challenged orally with 50,000 eggs and two pairs were killed according to the same schedule as described above. These mice given prior stimmulating infection before being given challenging infection were referred to as immunized mice. Two uninfected mice (controls) were killed on day 1 and other 2 mice

on day 4.

All the animals were killed by cervical dislocation. The intestine from pylorus to the end of the subsequent 20 cm long segment was removed immediately after death and fixed in either 10% formalin or Carnoy's fixative. All the intestinal sample from each animal was cut into round slices, embedded in the paraffin block, sectioned 6 μm thick and stained routinely with haematoxylin and eosin. Some slides were stained with PAS, Azan or Van Gieson staining techniques.

The assessment were made on the following characteristics: presence and location of *H. nana* larvae; developmental stages of the larvae; morphology of the intestinal mucosa and submucosa; prevalence of leucocytes and other cellular components in the intestinal mucosa and submucosa.

The five developmental stages of larvae as proposed by Voge (1964) for *H. microstoma* were employed for the assessment of larval growth observed in our experiment.

Results

The results of the time course observations on the morphology of the intestinal villi infected with *H. nana* larvae are recorded in Table 1 where the intensities of the histopathologic changes around the larvae have been arbitrarily quantitated according to the following criteria: − ; no differeces in histological picture as seen in the uninfected mice, ± ; moderate reactions, some sections lacking any detectable changes, + ; weak but clearly detectable reactions, and ++ ; strong reactions. Growth of oncospheres to the cysticercoids in the intestinal villi and histopathological features of the tissue around the larvae are shown in Figs. 1–18.

Primary infection. Sections of intestinal

Table 1 Development of *H. nana* larvae and the histopathologic reactions of the intestinal villi of mice following primary and secondary infections with *H. nana* eggs

	Hours after infection	Developmental stage of larvae[1]	Mononuclear cells	Neutrophils	Eosinophils	Edema	Nuclear debris
Primary infection	4	1	−[2]	−	−	−	−
	8	1	±	−	+	−	−
	16	1	+	±	±	−	−
	24	1	+	+	±	±	−
	48	2	+	+	±	+	−
	72	3, 4	+	++	+	+	+
	96	5	+	++	+	+	+
Secondary infection	4	1	+	+	−	+	−
	8	1	+	+	+	++	++
	16	1	±	++	++	++	++
	24	1	±	++	++	++	++
	48	−	−	−	−	−	−
	72	−	−	−	−	−	−
	96	−	−	−	−	−	−

1) The growth of the larvae was divided into five stages as described by Voge (1964).

2) The intensities of histopathologic changes have been quntitated according to the following criteria: − ; no differences in pathological picture as seen in the uninfected mice, ± ; moderate reactions, some section lacking any detectable changes, + ; week but clearly detectable reactions, and ++ ; strong reactions.

tissue taken 4, 8, 16 and 24 hours after primary infection evidenced that the oncospheres reached the lamina propria of the villi as early as 4 hours after infection (Fig. 2) and they grew into a spherical ball of cells during the next 20 hours (stage 1, Figs. 3–5). A small number of mononuclear cells was noted around the oncosphere at 16 hours after infection (Fig. 4) and there was a mild infiltration of neutrophils and mononuclear cells adjacent to the larvae at 24 hours of infection (Fig. 5). At 48 hours of infection an eccentric and rounded cavity was formed in the half of the larval body (stage 2, Fig. 6). At this time, the host-tissue reaction consisted of a small number of neutrophils around the growing larva, and some eosinophils were occasionally observed in the lesion. The formation of the outline of the suckers and rostellum, and the invagination of the anterior part of the larval body occured at the end of 72 hours after infection (stage 3 and 4, Figs. 7, 8). The cysticercoids of which the rostellar hooks and scolex musculature appeared to reach full growth were observed in the villi at 96 hours after infection. The cellular infiltration reached a zenith during 72 and 96 hours after infection. A considerable number of neutrophils, some eosinophils and mononuclear cells were observed around the larvae and throughout the villus. The degenerating cells with darkly stained nuclear debris appeared in the lesion around the larvae and edema was also prevalent throughout the villus. Figs. 2–8 show that, despite the presence of rapidly growing larvae and gradual increase of the infiltration of inflammatory cells, the intestinal mucosa preserved their general morphology (cf. Fig. 1) until the end of 72 hours after infection. At 96 hours after infection, however, the hight of mucosal villi was variable (Figs. 9, 10). In most cases the villi with mature cysticercoids were shortened and more dilated than normal, and the epithelium near the larvae usually consisted of rounded columnar cells often with an indistinct brush border.

Secondary infection. It was noted that only a small number of oncospheres were able to invade into the intestinal villi of immunized mice though quantitative assessment was not done in the present study. Although the egg dose level of secondary infection (50,000 eggs) was five times larger than that of primary infection, the number of larvae found in the tissue sections from immunized mice was markedly smaller than that found in those from nonimmunized mice. However, observations of the intestinal tissues taken 4, 8, 16 and 24 hours after secondary infection showed that a few oncospheres derived from secondary infection could enter the intestinal villi of immunized mice as early as 4 hours after infection (Figs. 11, 12) and they increased somewhat in size during the next 20 hours (stage 1, Figs. 13–18). These larvae die not show any further development. None of the larva were found in section of intestine taken 48, 72 and 96 hours after secondary infection.

The histopathological reactions of the intestinal tissue during the first 24 hours of secondary infection were characterized by its earliar initiation and greater severity as compared with that of primary infection. The first sign of infiltration of neutrophils and some mononuclear cells around the larva was noted 4 hours after infection (Figs. 11, 12). A small granulomatous nodule with the neutrophils and eosinophils as the dominant cells was rapidly developed around the larva within the next few hours (Figs. 13, 14), and this reaction reached a peak at 16 and 24 hours of infection (Figs. 15–18). Mucosal edema was evident and nuclear debris was frequently observed around the larva. The gross abnormalities in the morphology of the villi also became evident at the early stage of infection. The intestinal villi with invaded oncospheres were usually short, stubby and irregular in shape (Figs. 13–18). The epithelial cells of these villi were often cuboidal, rounded or flattened, and there was an occasional sloughing of the epithelial cells (Fig. 16). The severe tissue reactions of the villi against the larva diminished very rapidly, and only a moderate number of mononuclear cells were noted in the lamina

propria of some villi 48 hours after secondary infection. The topography of the villi at 72 and 96 hours of secondary infection appeared quite normal.

Discussion

Several investigators have suggested a possibility that the oncosphere of *H. nana* is strongly immunogenic and the protective immunity to challenge infections may be thus directed against this particular organism before or shortly after penetration into the intestinal mucosa of the previously infected mice (Bailey, 1951; Di Conza, 1969; Furukawa, 1974; Ito, 1975). Ito and Yamamoto (1976) presented evidence to support this possibility and suggested that, in immunized mice, resistance in both the intestinal lumen and villi take a share in the rejection of the larvae. The results presented in this paper indicated that the oncospheres in nonimmunized mice developed to mature cysticercoids in a similar manner to that described previously by Bailey (1951). In immunized mice, however, only a small number of oncospheres were able to penetrate into the intestinal villi and none of them showed any further development beyond stage 1. These results seem to provide further evidence for the observations of the previous workers.

A marked inflammatory reaction was observed around the invaded larvae in the intestine of both nonimmunized and immunized mice. However, the histological changes differed considerably with each other. In nonimmunized mice, the infiltration of a small number of neutrophils was noted by 24 hours after primary infection, indicating that the acute stage of inflammation had been initiated. This reaction with predominant infiltration of neutrophils and some eosinophils reached a peak 72 and 96 hours after infection. In immunized mice, the second infection provoked an acute inflammatory reaction as early as 4 hours after challenge infection. The inflammatory reaction reached a zenith between 16 and 24 hours after challenge infection and in such cases a considerable number of neutrophils and eosinophils formed a small granulomatous nodule around the path of oncospheres. The infiltration of eosinophils and the changes in topography of the villi also appeared to be more remarkable in the immunized mice. The inflammation diminished rapidly during the next 24 hours and no larvae was found in the intestinal tissue thereafter. It is apparent therefore that the inflammation in immunized mice was initiated sooner, developed a more rapid course and was more severe than that in nonimmunized mice. These results are suggestive that the inflammation observed in nonimmunized mice would be a nonspecific reaction, giving no harmful effect on the larval development. On the other hand, the inflammation in immunized mice would be an accelerated and intensified reaction, closed associated with the acquired immunological responses (allergic inflammation), which results in a complete expulsion of the oncospheres from the immunized mice.

It has been reported that infections of adult *Raillietina cesticillus* in the intestine of fowl caused mononuclear cell infiltration in the tissues around the worm scolices and such an infiltration was more prominent in secondary infections (Gray, 1973, 1976). An inflammatory reaction with prominent infiltration of neutrophil and eosinophils in the bile-duct of mice infected with adult *Hymenolepis microstoma* has been reported by several investigators (Lumsden and Karin, 1970; Sandborn *et al.*, 1970; Gleason, 1971). However, no comparable studies have been made as yet on the histopathology of larval cestode infections. The result of the present study suggests that the inflammatory reactions to *H. nana* larvae differ from the reactions to adult cestodes in that the initiation and development of inflammatory reaction to *H. nana* larvae is unexpectedly rapid. This difference appears to become more apparent at the time of secondary infection. The probable reason for this difference would be that the metabolic by-products of *H. nana*

oncospheres may be very iritative. It may also be due to the fact that the oncospheres are able to penetrate into the intestinal villi very rapidly (Miyazato *et al.*, 1977), and that almost all of these larvae do not migrate from the lamina propria of the villi after penetration.

It should be mentioned that little experimental attention has been given to the effect of host immunity on the eggs or on the newly hatched oncospheres in the intestinal lumen. Several investigators have suggested that the intestinal epithelium of the immunized mice becomes a serious barrier to the invasion of *H. nana* oncospheres (Bailey, 1951; Weinmann, 1966). However, we found that the penetration of *H. nana* oncospheres into the intestinal villi was very rapid and the host tissue destruction by the oncospheres was not merely enzymatic but physical as well (Miyazato *et al.*, 1977). These findings seem to give a little doubt as to whether the intestinal epithelium can in fact act as an intestinal barrier. Rather, it is likely that the majority of the hatched oncospheres in the intestinal lumen of immunized mice become inert or are killed by a specific immunological reaction.

Summary

Observations were made on the larval development and histopathology of the small intestine of mice which were given primary and secondary infections with *Hymenolepis nana* eggs. In primary infection, the oncospheres developed into mature cysticercoids at 96 hours of infection. An acute stage of inflammation with predominant infiltration of neutrophils and some eosinophils was initiated around the larvae 24 hours after infection and reached a peak from 72 to 96 hours after infection. In secondary infection, only a small number of oncospheres were able to penetrate into the intestinal villi of mice, and these larvae did not show any further development beyond stage 1. No larvae was found in the intestinal tissues 48, 72 and 96 hours after secondary infection. An acute stage of inflammation was noted 4 hours after infection and reached a zenith from 16 to 24 hours after infection. In such cases, a small but distinct granulomatous lesion consisting of neutrophils and eosinophils was formed around the oncosphere. The inflammation rapidly waned thereafter. An important protective role of the host immunity which was probably operated in the intestinal lumen, but not in the intestinal tissue, of previously infected mice was discussed.

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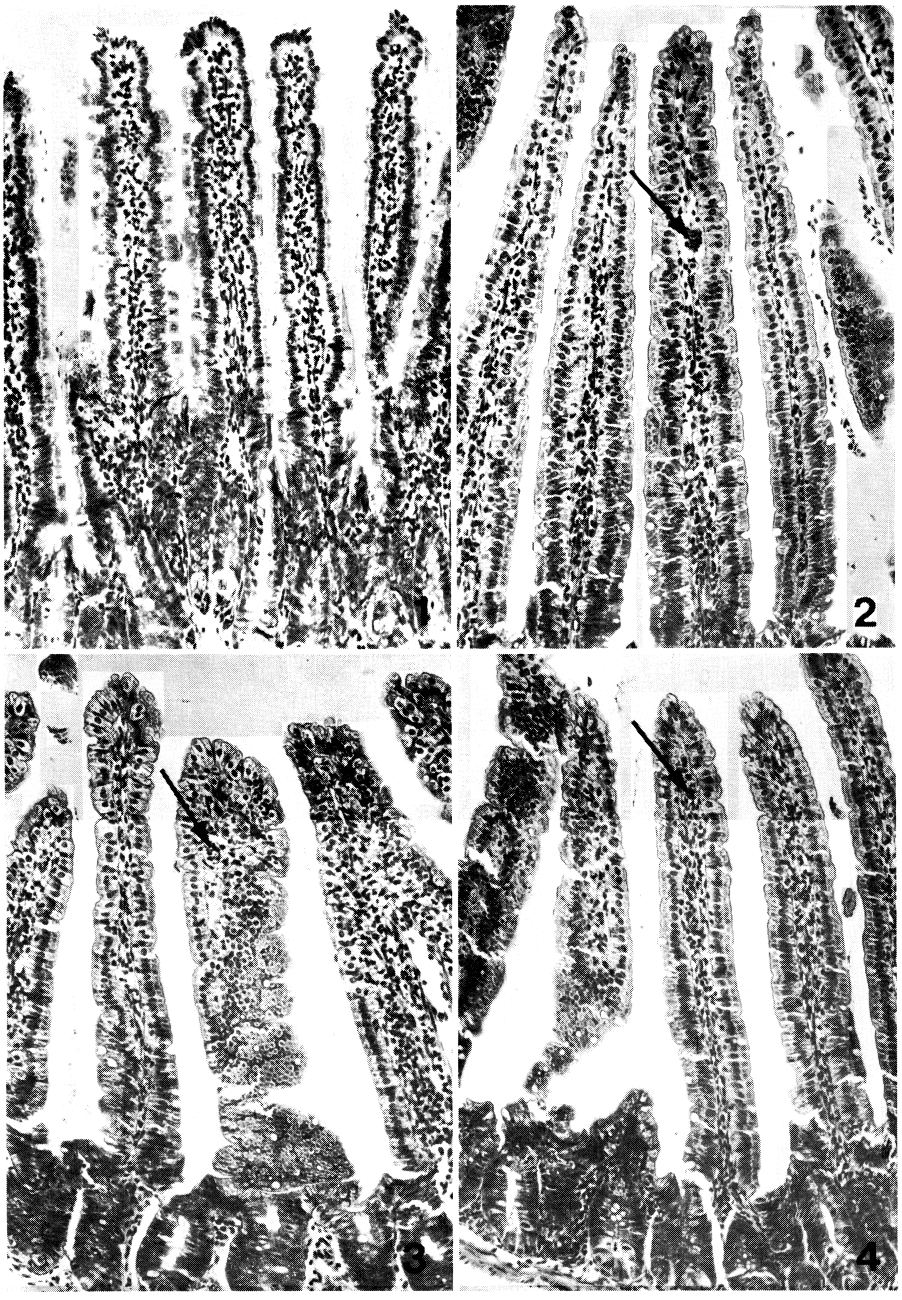
小形条虫の初感染および再感染時における幼虫の発育とマウス腸管の病理組織学的反応

宮里　昴　古川忠明　井上朝義
（近畿大学医学部寄生虫学教室）

マウスの腸絨毛内における小形条虫幼虫の発育と，宿主の感染局所の病理組織学的反応を検討するために，初感染群および再感染群のマウス腸管を組織学的に検索した．初感染群では感染後96時間以内に擬嚢尾虫の発育が完了した．感染24時間以後より宿主の組織に軽度の非特異的と思われる炎症反応が見られた．すなわち，幼虫の周辺から絨毛の基部にかけて，少数の好中球と好酸球の浸潤および軽度の浮腫が認められ，また感染72時間および96時間後にはこの炎症反応が若干強くなつた．再感染群では，感染後4～24時間目の組織より，極めて少数ではあるが六鉤幼虫が検出され，再感染由来の幼虫の一部が宿主の組織内に侵入し得ることを確認した．しかし，これらの幼虫はほとんど発育せずに死滅するものと思われ，感染48時間以後になると幼虫の検出は困難となつた．宿主の炎症反応は，感染16～24時間後に最も強く現われ，粘膜固有層全体に好中球，好酸球，単核細胞の浸潤や浮腫が見られるとともに，幼虫を中心とする小さな虫体結節状の病変が認められた．これらのアレルギー性と思われる炎症反応は，組織中の幼虫の消失に伴つて速やかに消退し，感染48時間目以後の組織像は正常に復した．再感染群の組織中より検出された幼虫が極めて少数であることから考えて，再感染由来の虫卵または幼虫の大部分が，腸腔内における免疫反応によつて不活化されるか，または死滅する可能性が示唆された．



Explanation of Figures

Fig. 1 Section of the intestinal mucosa of uninfected control mouse. Note the tall villi covered with tall columner epithelial cells. The lamina propria of the villi is moderately cellular. HE. ×140.

Fig. 2 Nonimmunized mouse 4 hours after infection with *H. nana* eggs. Note an invaded oncosphere (arrow) without cellular infiltration. HE. ×140.

Fig. 3 Nonimmunized mouse 8 hours after infection. Arrow indicates the invaded oncosphere. HE. ×140.

Fig. 4 Nonimmunized mouse 16 hours after infection. The lamina propria with invaded oncosphere (arrow) is more celluar than uninfected mouse. HE. ×140.

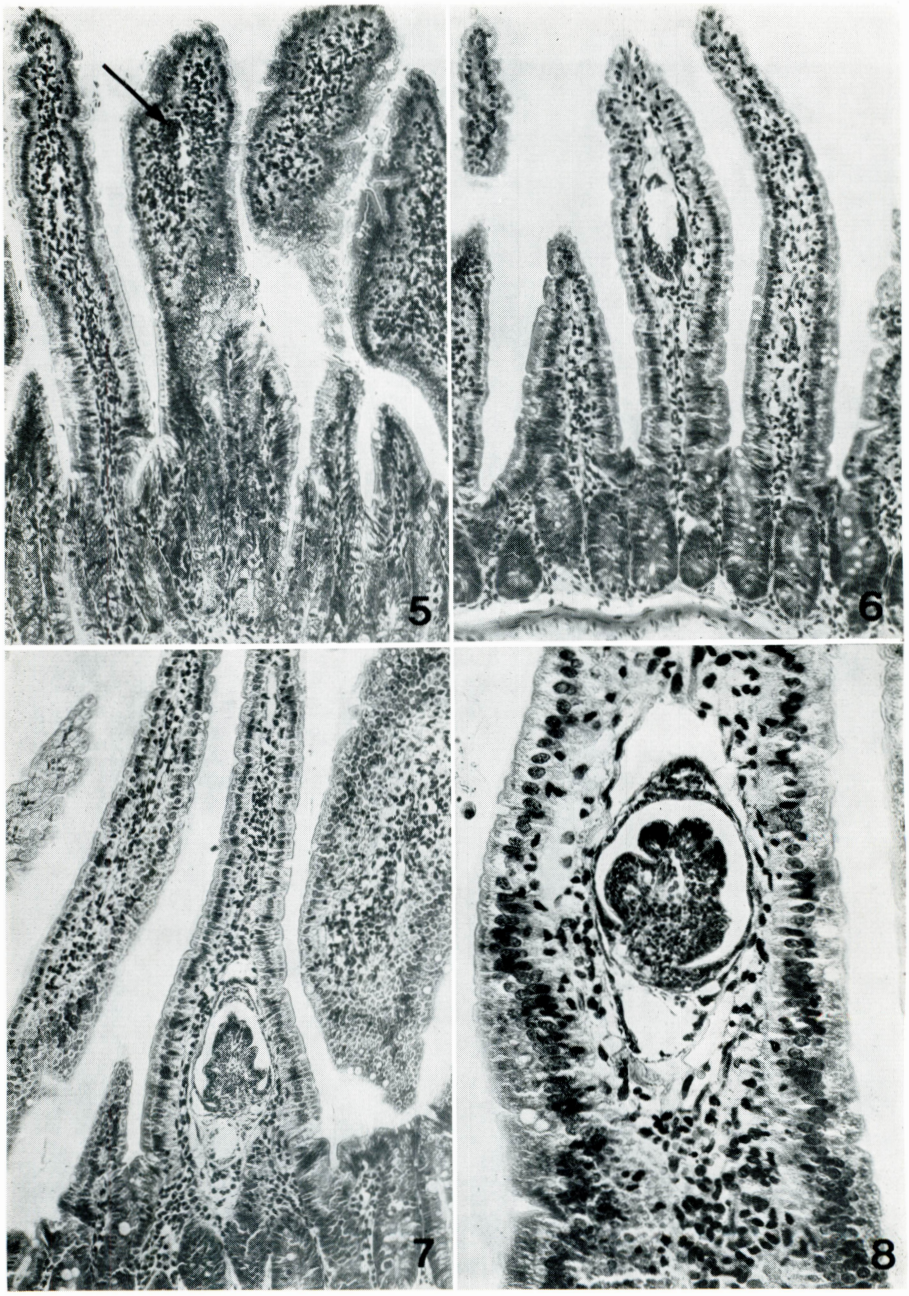
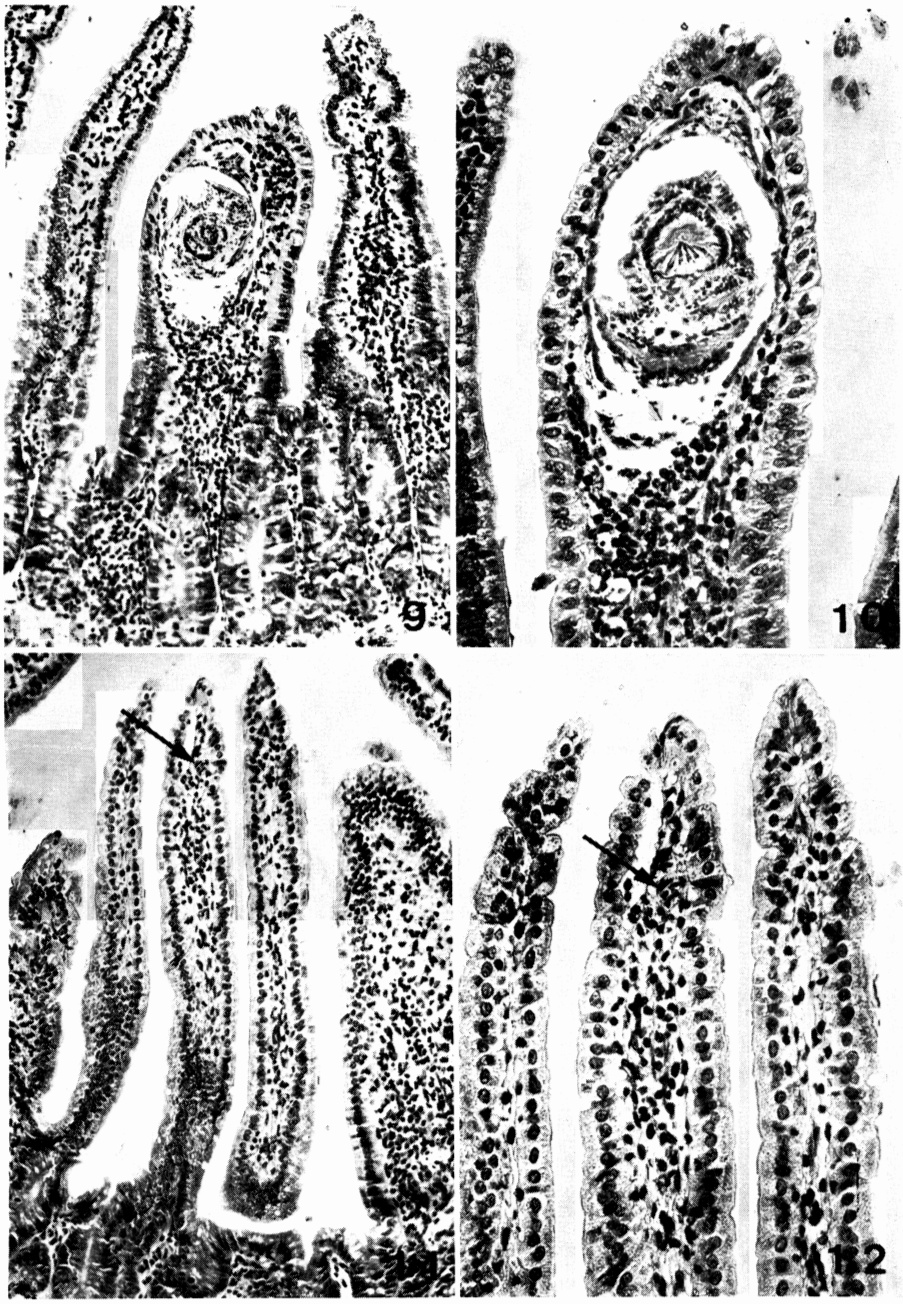


Fig. 5 Nonimmunized mouse 24 hours after infection. The infiltration of mononuclear cells and neutrophils around the larva (arrow) is noted. The oncosphere increases somewhat in size and consists of darkly stained packed cells. HE. ×140.

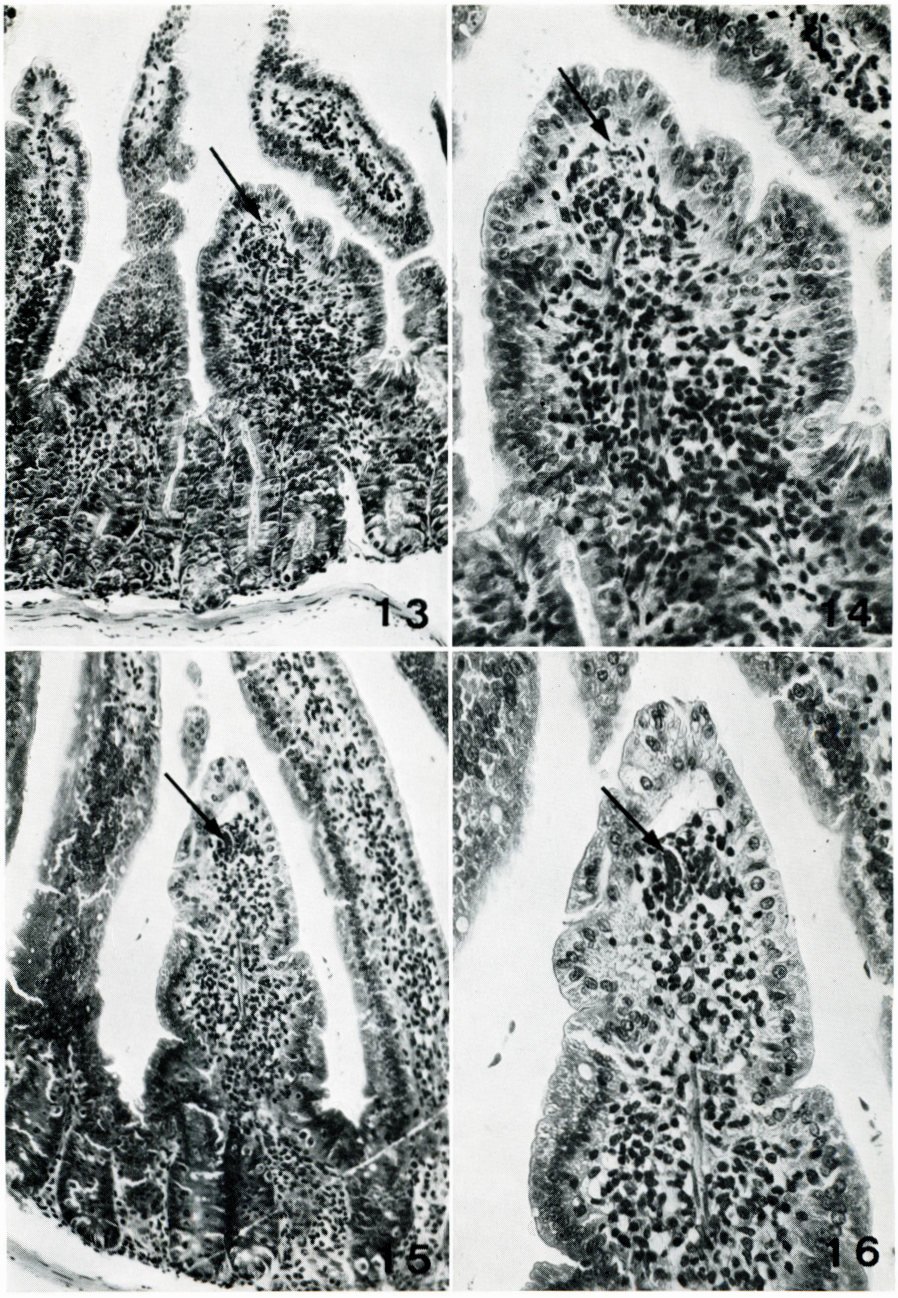
Fig. 6 Nonimmunized mouse 48 hours after infection. The larva has an eccentric and round cavity. A small number of mononuclear cells and neutrophils are seen in the basal portion of the villus. HE. ×140.

Figs. 7 and 8 Nonimmunized mouse 72 hours after infection. The anterior part of the larval body with the rostellum and suckers is invaginated into the cavity. The infiltration of neutrophils, mononuclear cells and some eosinophils is evident around the larva and through out the villus. HE. Fig. 7 ×140, Fig. 8 ×280.



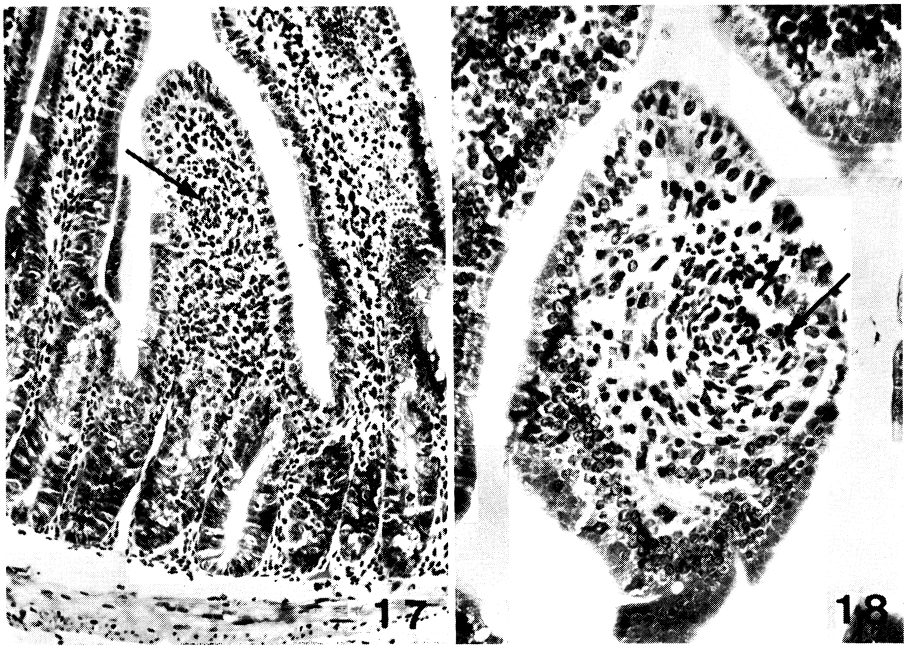
Figs. 9 and 10 Nonimmunized mouse 96 hours after infection. The growth of the cysticercoid is completed. Many neutrophils and some eosinophils are prevalent throughout the villus. The villus with the larva is short and stubby in shape. Note that the villous epithelium around the larva is composed of flattened cuboidal cells. Sloughing of the epithelial cells is noted at the tip of the villus. HE. Fig. 9 ×140, Fig. 10 ×280.

Figs. 11 and 12 Immunized mouse 4 hours after infection. Arrow indicates the invaded oncosphere. The lamina propria of the villus is only slightly cellular, but some mononuclear cells and neutrophils are attached to the oncosphere. HE. Fig. 11 ×140, Fig. 12 ×280.



Figs. 13 and 14 Immunized mouse 8 hours after infection. The infiltration of neutrophils, eosinophils and mononuclear cells is evident throughout the villus. A small granulomatous lesion is formed around the larva (arrow). The villus is short and somewhat convoluted. HE. Fig. 13 ×140, Fig. 14 ×280.

Figs. 15 and 16 Immunized mouse 16 hours after infection. The infiltration of inflammatory cells is intense. The degeneration and sloughing of the epithelial cells of the villus is evident especially at the tip of the villus. The larva (arrow) shows some increase in the number of the cells as compared with that shown in Figs. 12 and 14. HE. Fig. 15 ×140, Fig. 16 ×280.



Figs. 17 and 18 Immunized mouse 24 hours after infection. The intense inflammatory reaction is comparable to that shown in Figs. 15 and 16. Note that the inflammation extends to the submucosal region of the villus. The granulomatous lesion around the larva (arrow) is clearly shown in Fig. 18, the oblique section of the villus. HE. Fig. 17 ×140, Fig. 18 ×280.