THE EMBRYONIC DEVELOPMENT AND GROWTH OF THE MOUSE NEMATODE,
ASPICULIURIS TETRAPTERA (NITZSCH, 1821)

A Thesis Presented for the
Degree of Master of Arts

BY

Mildred Ann Hardie, B.A.

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Approved by:

J.M. Miller
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INTRODUCTION

The nematode, *Aspiculuris tetraptera*, is a common parasite of the laboratory mouse. Its site of infection is the lumen of the large intestine just posterior to the caecum. The worm is small, female 2.6-4.75 μ, male 2-2.6 μ (Yorke and Maplestone, 1926). The parasite is closely related to the human pinworm, *Enterobius vermicularis*, being placed in the same subfamily, *Oxyurinae*.

A high percent (approximately 90%) and a high incidence of infection (approximately 80) were found in laboratory mice of the Department of Zoology at The Ohio State University. These mice, which were kept by the department for genetic experiments, were not purposely infected, but acquired their infection naturally. The high percent and incidence of infection seemed to indicate a relatively simple type of life cycle patterned somewhat after that of the human pinworm. The author has attempted to determine certain parts of this cycle, especially the details of development and hatching of the egg in artificial media.

Development of eggs, hatching, and growth of larvae in artificial media may be accomplished rather easily with free-living forms and with those parasitic species that have free living stages; however, it is much more difficult with species that are dependent upon a host throughout their entire life cycle. A certain amount of research with this latter type of life cycle has been done on various species of the *Oxyuridae* by Philpot (1934), and on the human pinworm, *Enterobius ver-
micularis by Kouri (1938).

The oxyurid type of life cycle is probably the simplest of any found among nematodes that are parasitic in vertebrates. In spite of this fact little has been done in working out the details of development until Philpot (1924) described and figured the eggs and larvae of several species of the Oxyuridae. He was successful in incubating the eggs of Aspiculuris tetraperta in artificial media, but could not cause the embryos to emerge spontaneously from the shell. By stirring the culture rapidly or by allowing it to dry and remoistening, the embryos could be freed to a certain extent. He was also able to press the embryo out under a cover slip after the shell of the egg had been softened by pepsin and trypsin. Because he could not cause the larvae to emerge spontaneously from their shells under artificial conditions, he completed the cycle by feeding mice, and later, recovering the larvae which had hatched in the host. Philpot obtained larvae from mice four hours, eighteen hours, forty-four hours, ninety hours, and one hundred and sixty-three hours after feeding them eggs which had been previously incubated six or seven days at 25°C. Larvae, eight, ten, fourteen, eighteen and twenty-three days' old were also recovered from the mice, and described. In this way he was able to show stages in the life cycle of the worm.

When the present work was undertaken, a preliminary survey was made to determine if larval forms of Aspiculuris were present in the mice. The greater number of worms recovered were adults, but various
larval stages were observed. During this survey it was discovered that the mice were infected with another nematode, *Syphacia obvelata*, which form is closely related to *Aspiculuris*, occupying a taxonomic position in a related subfamily, *Syphaciinae*, of the family *Oxyuridae*. These two species, which are much alike in appearance, frequently occur together and inhabit the same general part of the digestive tract. It was necessary to differentiate *Aspiculuris* from *Syphacia* in order to separate one from the other, since the eggs of *Aspiculuris* only were desired. While the two forms are often confused, they may be readily distinguished by a few very apparent morphological variations which are listed at this time. A comparison of the characters listed in the chart with the figures of Plate 1 will illustrate many of the differences of the two worms.
# CHART I

**Aspiculuris tetraptera** compared with **Syphacia obvelata**

<table>
<thead>
<tr>
<th></th>
<th>Aspiculuris tetraptera Plate I</th>
<th>Syphacia obvelata Plate I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size of Worm</strong></td>
<td>2-2.6 mm. ♂</td>
<td>1.3 mm. ♂</td>
</tr>
<tr>
<td></td>
<td>2.6-4.75 mm. ♀</td>
<td>3.5-5.7 mm. ♀</td>
</tr>
<tr>
<td><strong>Cervical alae</strong></td>
<td>Very broad</td>
<td>narrow</td>
</tr>
<tr>
<td><strong>Body form ♀</strong></td>
<td>body short-tail conical</td>
<td>body extended posteriorly ending in a long pointed tail</td>
</tr>
<tr>
<td><strong>Body form ♂</strong></td>
<td>tail conical</td>
<td>bent posterior extremity conspicuous spicule</td>
</tr>
<tr>
<td><strong>Esophagus</strong></td>
<td>oval esophageal bulb</td>
<td>spherical esophageal bulb</td>
</tr>
<tr>
<td><strong>Uterus</strong></td>
<td>extends posterior of the anus</td>
<td>does not extend posterior of the anus</td>
</tr>
<tr>
<td><strong>Size of Egg</strong></td>
<td>symmetrical</td>
<td>asymmetrical</td>
</tr>
<tr>
<td></td>
<td>84-90 μ in length</td>
<td>110-142 μ in length</td>
</tr>
<tr>
<td></td>
<td>34-40 μ in diameter</td>
<td>30-40 μ in diameter</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

The eggs used in these experiments were obtained from specimens recovered from mice that had been chloroformed. The worms were removed from the host, and placed in concavity slides in various culture media. Some of the worms were macerated, and the entire worm left on the slide. Others were punctured, the eggs removed from the uterus, which was extruded through the puncture, and the remainder of the worm discarded.

A series of preliminary tests with various liquid culture media showed that the eggs of the parasite remained viable for six days in tapwater and also in various dilutions of normal salt solution. Because it was simpler to use, the author's first experiments were with tapwater as a liquid culture medium. Later experiments included other media.

In order to determine a method of hatching the eggs, and therefore to ascertain the life cycle, the author had to be guided by what seemed to be the natural cycle of the worm in the mouse. There seemed to be several possible ways in which the mouse acquired the infection in nature. These were:

1) The eggs might incubate and hatch outside of the body of the host, and the larvae be ingested by the mouse. If this were the natural method of infection, the eggs would be naturally be submitted to external environmental conditions during the time that they incubated and hatched. To test this possible method
of infection it was necessary to attempt to incubate and hatch the
eggs in tapwater at room temperature, thereby approximating the
natural thermal environment of the mouse.

This was done by placing unincubated eggs in tapwater in a
drop-culture slide. The slide was then placed in a moist chamber,
and removed every 24 hours for examination under a microscope.
Ordinary laboratory dessicators were converted into moist chambers
for incubating the eggs at room temperature. Several layers of
blotting paper were placed in the bottom of the dessicator, and water
added to this.

It was found that incubation of the eggs proceeded for only 48
hours, at the end of which time the embryo had taken on the appear-
ance of a tiny larva (Plate II, figure 4). Further incubation did
not take place, and the egg did not hatch.

This was repeated several times with tapwater and with other
media, such as tapwater plus mucosa scrapings, distilled water,
and normal saline. Distilled water and normal saline had a detri-
mental effect on the eggs, and the majority of them failed to show
any development; however, those cultured in tapwater plus mucosa
scrapings incubated to the 48 hour stage. These failed to develope
beyond this point, and so did not hatch.

Failure of the eggs to incubate past the 48 hour stage and
hatch at an environmental temperature seemed to indicate that the
that there was no free larval stage. The author concluded that
the mouse did not acquire the infection by ingestion of a lar-
vval form.

(2) It seemed possible, and in fact very probable, that the
mice acquired their infection by ingesting eggs. If infection
were acquired in this manner there were two possible variations
both of which had to be tested. These two variations were:

(a) Variation 1.—It seemed possible that the egg of the
parasite might be ingested as an undeveloped egg, the in-
cubation starting only after it had been submitted to the
body temperature of the mouse. If this condition of in-
cubation were the correct one, it was evident that the
development and hatching of the egg would have to be rapid,
since both would have to occur before the eggs passed the
length of the mouse intestine. To test this possibility
it was necessary to submit unincubated eggs in a culture
medium to a temperature approximating that of the mouse.
Therefore, the eggs were recovered, immediately placed
in culture in a moist chamber, and allowed to incubate at
98°F. Coplin staining dishes were converted into moist
chambers for the incubation of the cultures at the ele-
vated temperature. A piece of heavy lead foil was bent
to form a stand on which to rest the slide, so that it
would not touch the water present in the bottom of the
dish. The cover of the dish was sealed on by means of
vaseline.

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Development proceeded rapidly at this temperature. Fully incubated eggs (Plate III, figures 3 and 4) were present after 48 hours of incubation. Although the embryos were very active, they did not succeed in freeing themselves from the shells. Therefore, the author concluded that infection was not acquired by the ingestion of unembryonated eggs.

(b) Variation 2.—It seemed possible that the egg of the parasite might be fully incubated outside of the body of the host, and later ingested as a fully incubated egg containing a larva. It was evident that if this condition prevailed in nature, the author might be able to get eggs to hatch in artificial media by first incubating them under ordinary environmental conditions, such as room temperature, and then transferring them to an elevated temperature approximating that of the mouse (e.g., 98°F.).

In order to test this possibility, eggs were removed from a worm, and placed in culture at room temperature. At the end of 48 hours tiny larval forms were present in the eggs. The cultures were then placed at a temperature of 98°F., approximating the body temperature of the mouse. The embryos completed their development at this temperature, and hatching occurred. Therefore, the author concluded that infection was acquired by the ingestion of embryonated eggs.

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Since under natural conditions the worm eggs which passed in the feces of the mouse might be exposed to a certain amount of drying before their ingestion by a mouse, attempts were made to produce similar conditions by dessicating the worm before placing the eggs in a liquid culture medium. Several worms were dried in the open for 24 hours before the eggs were placed in tapwater. After 48 hours of incubation in tapwater at room temperature, the egg culture was put in a moist chamber in a water bath at 98°F. The hatching of the eggs had been completed by the end of the sixth day, and hatching did not occur after 144 hours of incubation. It was concluded that the eggs will withstand a period of drying, at least 24 hours and perhaps more (dessication of the eggs for a greater number of hours was not attempted by the author), without losing their ability to develop and hatch. This being true, such eggs would produce an infection if ingested by a mouse.
## CHART II

<table>
<thead>
<tr>
<th>Distilled Water</th>
<th>Tapwater</th>
<th>Tapwater plus Mucosa Scrapings</th>
<th>Normal Saline</th>
<th>0.5 Normal Saline plus Mucosa Scrapings</th>
<th>0.25 Normal Saline plus Mucosa Scrapings</th>
<th>0.25 Normal Saline plus Feces from the Large Intestine and Caecum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature</td>
<td>No Hatching</td>
<td>No Hatching</td>
<td>No Hatching</td>
<td>No Hatching</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
</tr>
<tr>
<td>Room Temperature 98°F</td>
<td>No Hatching</td>
<td>No Hatching</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
</tr>
<tr>
<td>Room Temperature 48 Hours to 98°F</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
</tr>
<tr>
<td>Room Temperature 72 Hours to 98°F</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
</tr>
<tr>
<td>Room Temperature 24 Hours to 98°F</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
</tr>
<tr>
<td>Room Temperature 120 Hours to 98°F</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
</tr>
<tr>
<td>Room Temperature 144 Hours to 98°F</td>
<td>Hatching 15th day</td>
<td>Hatching 15th day</td>
<td>Hatching 15th day</td>
<td>Hatching 15th day</td>
<td>Hatching 15th day</td>
<td>Hatching 15th day</td>
</tr>
<tr>
<td>Room Temperature 216 Hours to 98°F</td>
<td>Hatching 12th day</td>
<td>Hatching 12th day</td>
<td>Hatching 12th day</td>
<td>Hatching 12th day</td>
<td>Hatching 12th day</td>
<td>Hatching 12th day</td>
</tr>
<tr>
<td>Room Temperature 288 Hours to 98°F</td>
<td>Hatching 16th day</td>
<td>Hatching 16th day</td>
<td>Hatching 16th day</td>
<td>Hatching 16th day</td>
<td>Hatching 16th day</td>
<td>Hatching 16th day</td>
</tr>
<tr>
<td>Room Temperature at Tapwater at 75°F to 98°F</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
<td>Hatching 6th day</td>
</tr>
</tbody>
</table>
RESULTS

THE EGG

The egg of *Aspiculuris tetragona* is symmetrically oval. It possesses three membranes which resemble those described by Jacobs (1940) for the eggs of various nematodes. Because the membranes appear similar to those described by Jacobs, the author has used the same terminology in designating the membranes of the egg of *Aspiculuris*. The inner membrane is the vitelline membrane; the outer, the protein membrane; and the middle, the shell proper.

In each egg there is a thinner place through which the larva emerges from the shell (Plates II, III, and IV). At this point only two membranes, the vitelline membrane and the protein layer, are apparent. Philpot (1924) described this area as lying on the antero-ventral portion of the egg, but the present author has been unable to distinguish dorsal and ventral surfaces of the egg, and has therefore described the area of emergence as lying on the antero-lateral portion of the egg shell. In some eggs a definite operculum appears to be present, since at the thinner portion of the shell, the inner membrane, is indented in the form of a cone, and the outer membrane, the protein membrane, is slightly everted, and the two appear to form a cap at this point. However, as the embryo develops, the cone-shaped indentation of the vitelline membrane disappears, and the resemblance of this area to an operculum is lost.
Most eggs which possessed such am area were not fertile; however, it was present in a few fertile eggs. Evidently this area is not necessary for development and hatching, since in those fertile eggs in which it was absent, development and hatching still occurred.

The eggs when removed from the uterus of the female measured 87.75-93 μ in length and 38.7-42 μ in diameter (average 90.2 μ by 40 μ). Several kinds of eggs were noted. Some contained a dark, homogeneous mass. A clear circle was discernible in a few eggs, and in these no dark substance was present. All eggs of these two types failed to show any degree of development by any method of incubation, and apparently none of them was fertile.

Some eggs contained a dark, irregularly shaped mass similar to a morula. These evidently were fertile since embryos usually developed in them (Plate II, figure 1). No definite cell structure was observed in these morula-like masses. Eggs of this type were present nearest the vulva in the upper part of the uterus, as would be expected of ripe eggs. These eggs were in the earliest stage recognizable as that of a developing embryo. Larvae developed from only this type of egg.

INCUBATION

Since, as already indicated, it had been found that hatching only occurred when eggs were incubated for 48 hours at room temperature (approximately 75°F.) before placing them at an elevated temperature (98°F.) (see Chart II), the discussion which follows is for the development and hatching of eggs incubated in this manner.
The eggs were removed from the worm, placed in culture in concavity slides, and the slides put in a moist chamber at room temperature. As already indicated, the eggs from the vulva of the worm contained an irregularly shaped mass when put in culture (Plate II, figure 1).

24 HOURS INCUBATION

After 24 hours incubation at room temperature, the mass within the egg had enlarged, and a more definite outline was present. The anterior end of the developing embryo was blunt, and the posterior and was slightly concical (Plate II, figure 2). No movement was apparent at this stage.

48 HOURS INCUBATION

Some of the eggs seemed to develop faster than others. After incubation for 48 hours most of the embryos extended the full length of the shell; however, some were slightly shorter (Plate II, figures 3 and 4). The uneven outline had disappeared, and the mass had become worm-like in appearance. Movement was noted at this time. This movement consisted of the embryo's rocking back and forth from side to side in the shell. After this period of incubation at room temperature, the eggs were put in moist chambers in an electric water bath, the temperature of which remained at 98°F., a temperature approximating that of the mouse. Eggs after ingestion by a mouse would be under thermal conditions simulated by the elevated temperature.
72 HOURS INCUBATION

An increase in the length of the embryo was noted after 24 hours of incubation at 96°F. Movement of the embryos was more pronounced, but no definite structural beginnings were evident (Plate III, figures 1 and 2). Variation in the length of these two embryos is again due to difference in rate of development. In some cultures one or two eggs hatched, and the larvae were found free in the medium. This was the exception, not the rule; however, in most cultures a very few of the eggs hatched on the fourth day. This precocious development and hatching is probably explainable by the fact that some eggs were in advanced stages of incubation (Plate II, figures 2, 3, and 4) when removed from the worm; however, no eggs in such advanced stages at the time of removal from the worm were observed by the author.

96 HOURS INCUBATION

The embryo had so increased in length that it was bent upon itself in the shell (Plate III, figures 3 and 4). Anterior and posterior ends were distinguishable, the anterior end being rounded, the posterior being pointed. The embryo moved almost constantly, twisting and turning within the shell. At times, it completely reversed, the anterior end leading in all movements. A few larvae were beginning to emerge from their shells. Very faint beginnings of gut differentiation were apparent at this time.
120 HOURS INCUBATION

Free larvae and eggs in the process of hatching were present in cultures incubated 120 hours. All embryonated eggs which hatched did so after 120 hours of incubation, and only in unusual cases did more eggs hatch. The larvae always emerge at the thinner portion of the egg shell. The rounded anterior end emerges first (Plate IV, figures 1 and 2), and the shell is often thrown about as an appendage by the movement of the larva. The free larvae measured 174.2 μ by 24 μ. The esophageal bulb was becoming distinct and a faint outline of the gut and esophagus was also discernible (Plate IV, figure 3.) Some movement was present, but lasted only a short period after complete emergence of the larva from the shell. The freed larvae ceased all movement, and, in a day or two, began to disintegrate.

The embryos in some eggs apparently died since movement ceased, and they failed to hatch. Embryos dying before the fourth day—before completion of 72 hours of incubation—disintegrated and a granular mass was left in the shell.

LARVAE

Because the larvae did not remain alive in the media used in culturing the eggs, attempts were made to cultivate them on other media. McCoy (1929) was successful in rearing hookworm larvae on pure cultures of bacteria grown in an agar medium. The entire life cycle of *Nesaplectana glaseri* was obtained on artificial media by Glaser (1931). This nematode was cultivated on standard meat infusion agar containing 1% dextrose and having a pH 7.4.
The type of food required by Aspiculuris larvae was not determined. They, like hookworm larvae, may require bacteria as food, in which case they must thrive on bacteria present in the intestine of the host. On the other hand, food in the form of carbohydrates or proteins may be absorbed by them from the material present in the intestine of the mouse. All of these possibilities were considered and tested by transferring the larvae to agar plates and incubating the plates at 98°F. The kinds of agar used were:

(a) Nutrient agar  
(b) 0.1% glucose agar  
(c) 0.5% glucose agar  
(d) 2% peptone agar

The presence of bacterial colonies on the plates indicated that bacteria were transferred with the larvae to the plates. These bacteria were not identified and their origin was not determined. Because some bacteria were transferred with the eggs from the intestine of the mouse to the culture slide, and, therefore, with the larvae from the culture slide to the agar plate, the author concluded that some of the colonies represented members of the bacterial population of the intestine of the mouse.

The larvae did not survive, and only the colonies of bacteria were present on the agar plates. No trace of the larvae could be found, and apparently they had disintegrated. The author was unsuccessful in rearing Aspiculuris larvae with artificial media, and
therefore, is unable at this time to demonstrate the larval stages of the life cycle.

INFECTION OF MICE

Philpot (1924) attempted to infect mice by feeding them with eggs containing immature embryos (incubated for one day at 25°C.). The mice were not infected, and the eggs were recovered unchanged from the caecum and rectum after four hours. An infection was obtained when the mice were fed with eggs which had been incubated for 6 or 7 days at 25°C.

In the present experiments, attempts were made to infect mice with Aspiculuris by feeding them with eggs which had been incubated in tap-water at room temperature for at least 48 hours. Larvae recovered sixteen days after the eggs were fed to the mice were positively identified as Aspiculuris (Plate V). Adult worms could be recovered after a period of three or four weeks. Infection of the mice by feeding them with eggs containing developing embryos showed that the life cycle of this worm is direct, and that no intermediate host is involved.
SUMMARY

The eggs of *A. tetraperta* can be successfully cultured in tapwater, 0.5 normal saline, 0.25 normal saline and various combinations of these substances with mucosa scrapings and with feces. A period of incubation at a reduced temperature, that is, at a temperature below the body temperature of the mouse, is necessary before hatching will occur at the higher temperature. This is to be expected since in nature the eggs, passed in the feces of the mouse, probably remain at environmental temperature for a period of time before being ingested by another mouse. Eggs cultured at only room temperature reach a certain stage of development (Plate II, figure 4), but fail to hatch. When the eggs are allowed to incubate at room temperature until this stage of development is reached, and are then exposed to a temperature of 98°F., which approximates the body temperature of the mouse, hatching occurs, and many larvae are found entirely free of their shells. The period of incubation at room temperature may be varied, but two days (48 hours) are usually necessary before a large proportion of the eggs will hatch at the higher temperature.

According to Philpot (1924) no infection was obtained by feeding mice with eggs containing immature embryos which had been incubated at 25°C. for one day. However, he found that infection followed in most cases when the mice were fed with eggs which had been incubated for six or seven days at 25°C. In the present experiments an infection was obtained by feeding mice with eggs which had been incubated...
at least two days (48 hours) at room temperature (75°F).

The life cycle of Aspicularis is direct. No intermediate host is involved. The mice acquire the parasites by ingesting eggs that have embryonated at least 48 hours, since eggs, to be infective, must have incubated this length of time attained the stage of development illustrated by figure 4 and have Plate II.

There is a definite correlation between the direct type of life cycle of Aspicularis tetrapecta and the high percent and high incidence of infection found in the mice. This simple type of life cycle, in which the infection is acquired by ingestion of the egg, accounts for the high percent and high incidence of infection, since the mice are constantly infecting and reinfecting themselves.
BIBLIOGRAPHY


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PLATES
PLATE I

Figure 1. *Aspicularis tetraptera*, anterior extremity, ventral view.
   (From Yorke and Maplestone, after Schulz.)

Figure 2. *Aspicularis tetraptera*, posterior extremity of female, lateral view, X 40. (Orig.)

Figure 3. *Aspicularis tetraptera*, male. (From Yorke and Maplestone, after Schulz.)

Figure 4. *Aspicularis tetraptera*, egg, X 150. (Orig.)

Figure 5. *Syphacia obvelata*, anterior extremity of female, lateral view, X 330. (After Yorke and Maplestone.)

Figure 6. *Syphacia obvelata*, posterior extremity of female, lateral view, X 215. (After Yorke and Maplestone.)

Figure 7. *Syphacia obvelata*. Egg, X 150. (Orig.)

Figure 8. *Syphacia obvelata*. Male, lateral view, X 75. (After Yorke and Maplestone).
PLATE II

Figure 1. Fertile egg from uterus of female *Aspiculuris*, X 440.

Figure 2. Egg after 24 hours' incubation at room temperature, X 440.

Figure 3. Egg after 48 hours' incubation at room temperature, X 440.

Figure 4. Egg after 48 hours' incubation at room temperature, X 440.
Figure 1. Egg after 72 hours' incubation, X 440.

Figure 2. Egg after 72 hours' incubation, X 440.

Figure 3. Egg after 96 hours' incubation, X 440.

Figure 4. Egg after 96 hours' incubation, X 440.
Figure 1. Egg with larvae emerging, 120 hours' incubation, X 440.

Figure 2. Egg with larvae emerging, 120 hours' incubation, X 440.

Figure 3. Larvae, X 440.
PLATE V

Larvae, recovered from mouse

16 days after infection.