# THE LIFE HISTORY OF *LEIDYNEMA APPENDICULATA* (LEIDY, 1850) CHITWOOD, A NEMATODE PARASITIC IN THE COCKROACH, *PERIPLANETA AMERICANA* (LINN.)

by

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# INTRODUCTION

Entozoa from insects, including a description of the nematode, Oxyuris diesingi, from the cockroach, Blatta orientalis, were first reported by Hammerschmidt in 1838. In 1847 he described another nematode, Oxyuris blattae orientalis, from the same host. Both nematodes were described by Leidy (1850); the first was referred to as Aorurus streptostoma gracile and the second as Aorurus thelastomum appendiculatum. Diesing (1851) described the first mentioned nematode and named it Anguillula macrurae. In 1871 Bütschli published anatomical descriptions of these nematodes.

The contributions by Galeb (1878), Magalhaes (1900), Pessoa and Correa (1926), Schwenk (1926, 1929) and Walton (1927) deal primarily with descriptions of new species or classification and redescriptions of old forms. Chitwood (1932) has dealt with the taxonomy of nematode parasites in Blattidae. He placed both of the above species in the family Thelastomidae Travassos, 1929, and the subfamily Thelastominae Travassos, 1920. Oxyuris diesingi has been transferred to the genus Hammerschmidtiella (Chitwood, 1932). Because Hammerschmidt's description of Oxyuris blattae orientalis appeared to be Oxyuris diesingi redescribed, it

becomes a synonym of Hammerschmidtiella diesingi and is also a synonym of Leidynema appendiculata (Leidy, 1850) Chitwood, 1932.

Yorke and Maplestone (1926), Sprehn (1932) and other helminthologists consider intestinal diverticulae as family characters. Chitwood employs it as a generic character. On the basis of this character Leidynema appendiculata should be transferred to the family Cruzidae Travassos, 1917, or to a new family. However, it cannot be denied that except for this difference the nematode has the characteristics of the family and genera to which it has been assigned and it may be undesirable to change this classification.

Most of the published work dealing with this group of nematodes is taxonomic or anatomical. In view of this it was considered desirable to make a study of the life history of these oxyurids, which are of common occurrence in the American cockroaches (Periplaneta americana) at Manhattan, Kansas. Although both of the species already mentioned were utilized in this investigation most consideration was given to Leidynema appendiculata.

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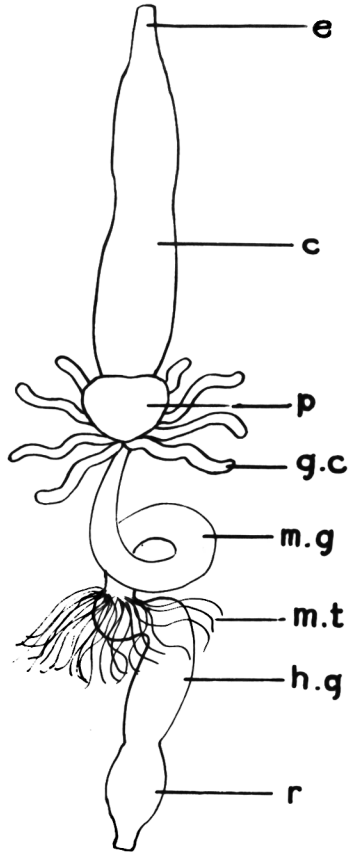
## MATERIAL AND METHODS

These parasitic nematodes are found in the hindgut of the cockroach (Text-fig. 1); they are most concentrated in the region of the Malpighian tubules. In isolating the nematodes, the intestines of P. americana were removed by one of the following procedures: the insect was decapitated and the digestive tract extricated by withdrawing with forceps the posterior abdominal segment and intestine. Another method (Ackert and Wadley, 1921) was to pin down the insect and slit longitudinally the ventral body wall. With the aid of needles and fine forceps the entire intestine was removed. The latter method was more satisfactory as the organs were less likely to be damaged; but, it was more time consuming. The part of the alimentary canal to be examined was placed on an ordinary slide. After adding a few drops of isotonic salt solution, the entire section of intestine




Text-figure 1. Showing digestive tract of Periplaneta americana. c, crop; g.c., gastric caeca; h.g., hindgut; m.t., Malpighian tubules; m.g., midgut; o, esophagus; r, rectum.

was laid open by a longitudinal slit or by teasing with needles. All the parts were examined under a dissecting binocular microscope, but when searching for larvae and eggs a compound microscope was used.

The nematodes were freed from the intestinal contents with the aid of Minuten insect pins and transferred to fluids in hanging drop or concave slides with the use of camels hair brushes and insect pins. The very small worms were often transferred by means of capillary pipettes.

## Egg Cultures

In preparing egg cultures two methods were used. By a method similar to that of Ackert, Graham, Nolf and Porter (1931) the extreme anterior end of a gravid female worm was excised and the body cavity contents pressed out. The uteri were separated from the other reproductive organs and placed in culture fluid where they were triturated to liberate the eggs. In the other procedure the female worms were placed in the culture medium where, perhaps due to unnatural conditions, they laid practically all their eggs. After completion of this process, which lasted 12 or more hours depending on the temperature, the worms were removed leaving a debris-free culture. The culture slides were placed in Petri dishes containing a few drops of water. When more

cultures were needed the culture slides were stacked in a large moist chamber.

## Technic of Slide Preparation

Perhaps the greatest handicap in this work was the failure to find satisfactory media for fixing, staining and mounting the nematodes. Some of the fixatives tried were Schaudinn's fluid, both hot and cold, Bouin's fluid with various modifications, hot alcohol, and Carnoy and Lebrun's fluid. Of these the last mentioned fixed with the least distortion, but it produced a 10 per cent shrinkage in the length and width of the worms. Little shrinkage occurred during alcoholic hydration and dehydration, when these processes were carried out slowly or by the drop method. In attempts to find a suitable stain for these nematodes practically all of the ordinary and many special dyes were tested, but none was found to be entirely satisfactory. In general, stains in alcoholic solutions as haemacalcium, paracarmine and Grenacher's carmine proved to be more satisfactory than those in aqueous solvents. Specimens stained in ordinary haematoxylins and other aqueous dyes, such as borax carmine, became badly distorted. The anilins were of little value in differentiating the anatomical structures for they stained all parts with equal intensity. Fortunately the cuticular and muscular layers of many of these nematodes are so clear

that most of the structures can be distinguished without the aid of pigments.

## Rearing Cockroaches

The P. americana for the experiments were obtained from buildings on the Kansas State College campus, Manhattan. A few Blattella germanica and Blatta orientalis were also used but on this campus both of these species were comparatively rare. The insects were caught by means of traps similar to those described by Marlatt (1928) and by Haber (1919). However, when the roaches were present in large numbers it was not difficult to collect them by hand. Until needed the specimens were kept in fruit jars and in one-gallon tin cans. To prevent the cockroaches from escaping the walls of the containers were given a thin coat of butter.

The food requirements of the roaches were very simple; a few drops of water and small morsels of table scraps were provided each week. There was little or no cannibalism when the roaches were kept well supplied with food. Under these conditions large numbers of cockroaches were maintained in each container for more than a year with very low mortality.

To rear uninfested roaches, oöthecae were collected

from the natural habitats of the insects and allowed to hatch in clean fruit jars. The brood capsules hatch in six months. Three or four months were required before the young roaches had attained a size sufficient for experimental use.

## Incidence of the Oxyurid Parasites at Manhattan, Kansas

The data for determining the incidence of oxyurid infestation was based on the examination of P. americana found under natural conditions. Most of the cockroaches were killed immediately after capture, but some were confined for a few days before examination. As shown in Table 1, males, females, young roaches and mature P. americana whose sexes were not recorded were examined for parasitic nematodes. All together 260 cockroaches were examined and of these 223 were infested with either one or both species of nematodes. Of the above 223 specimens 44.7 per cent were infested with L. appendiculata, 23.3 per cent with H. diesingi and 31.8 per cent with both species of worms. It was found that hosts collected from the same habitat, such as a room or box, usually had a predominance of the same species of parasites. The results also show that 79.5 per cent of the males, 87.5 per cent of the females and 94.2 per cent of the immature roaches contained these parasites.

Table 1. Incidence of Cockroach Oxyurids

| | Total number | Number infested | Percent infested | Number uninfested | Number with L. appendiculata | Number with H. diesingi | Number with H. diesingi and L. appendiculata |
|---|---|---|---|---|---|---|---|
| Males | 98 | 78 | 79.5 | 20 | 36 | 14 | 21 |
| Females | 80 | 70 | 87.5 | 10 | 22 | 17 | 28 |
| Immature | 52 | 49 | 94.2 | 3 | 20 | 13 | 9 |
| Adults, sex not recorded | 29 | 25 | 84.1 | 4 | 12 | 3 | 6 |
| Total | 260 | 223 | 85.8 | 37 | 90 | 47 | 64 |
| Percentage of total | | | | 14.2 | 44.7 | 23.3 | 31.8 |

There was no apparent difference between the males and females which would account for the higher infestation in the latter. The fact that growing animals normally require more food than adults may explain the higher infestation of the immature roaches. The difference in infestation of the young and the mature roaches may be due to the acquired resistance of the adults. There also appears to be some natural resistance to the parasites for about 15 per cent of the cockroaches in the same habitat were not infested.

In determining the sizes of the infestations (Table 2) only the mature nematodes were recorded. The range of infestation was from one to 21 with an average of 3.8 worms per cockroach. In the case of the male and female P. americana there appears to be a high correlation between incidence and size of infestation, but this correlation does not hold for the immature roaches in which the incidence of nematodes was high and the size of infestation low. Ackert and Wadley (1921) obtained similar results from nematodes parasitic in the cricket, Gryllus assimilis Fab. They found that 85 per cent of the adults, 70 per cent of the males, and 90 per cent of the female crickets were infested and that the females contained a larger number of the parasites.

Table 2. Size of Infestations with Mature Nematodes

| Number of Oxyurids | Cockroaches | | | | |
|---|---|---|---|---|---|
| | Males | Females | Immature | Sex not recorded | Total |
| 1 | 15 | 8 | 10 | 1 | 34 |
| 2 | 12 | 8 | 10 | 1 | 31 |
| 3 | 11 | 9 | 6 | 4 | 30 |
| 4 | 9 | 9 | 4 | 2 | 24 |
| 5 | 2 | 8 | 1 | 1 | 12 |
| 6 | 2 | 5 | 1 | | 8 |
| 7 | 1 | 7 | 1 | | 9 |
| 8 | 1 | 4 | | | 5 |
| 9 | | 1 | | 1 | 2 |
| 10 | 1 | 1 | | | 2 |
| 11 | | 1 | 1 | | 2 |
| 13 | | 1 | | | 1 |
| 16 | | 2 | | | 2 |
| 19 | 1 | | | | 1 |
| 20 | | 1 | | 1 | 2 |
| 21 | | 1 | | | 1 |
| Average infestation per roach | | | | | |
| | 3.8 | 5.1 | 2.7 | 5.1 | 3.8 |

## Host-Parasite Relationship

Certain nematode parasites have marked effects on their hosts, while others produce no apparent injury. Glaser and Wilcox (1918) attributed the high mortality of grasshoppers, Melanoplus atlanis, to infection with the mermithid, Mermis nigrescens. Cobb, Steiner and Christie (1923) stated that a mermithid, Agamermis decaudata, killed or hindered normal growth of many grasshoppers in which it was parasitic. Uvarov (1928) considered worm parasites effective in controlling locusts and grasshoppers. Cobb (1921) reported a nema parasite harmful to the cucumber beetle. Sharga (1932) gave evidence to show that the nematode, Tylenchus aptini, parasitic in thrips produces degeneration of the ovaries. Goodey (1930-31) found that the frit-fly, Oscinella frit, was rendered sterile due to the action of a nematode, Tylenchinema oscinella. Studies by Glaser (1932) show that Neoaplectana glaseri, a nematode, destroyed the grubs of the Japanese beetle by feeding upon the tissues. Christie (1931) reported oxyurid parasites from coleopterous larvae. Oldham (1930) found that elm-bark beetles were sterilized by parasitic nematodes. Merrill and Ford (1916) reported that the nematode, Diplogaster acrivora, in no way injured termite hosts. Ackert and Wadley (1921) concluded that Cephalobium

microbivorum failed to seriously affect its host, Gryllus assimilis. Periplaneta americana is apparently not affected by its oxyurid parasites. Specimens which were heavily infested were maintained in captivity for over a year without any apparent harm. Transverse sections of the insect's intestine show that the nematodes were usually distributed throughout the hindgut. Often they were found within the crypts, but never within the intestinal tissues. There was no evidence of tissue destruction. All observations seem to indicate that the health, fertility and activity of the heavily infested cockroaches were comparable with those of the non-parasitized specimens. Nematodes, therefore, appear to be of no economic importance in the control of P. americana.

## Anatomy of the Adult Worms

A detailed description of the structures of H. diesingi and L. appendiculata was published by Bütschli (1871). The illustrations by Galeb (1878) are very diagrammatic but they give the distinguishing features of the two nematodes. The typical female Hammerschmidtiella diesingi is characterized by an anterior esophageal pseudobulb; absence of an intestinal diverticulum; posterior intestine without a loop; vulva in anterior part of body. On the other hand, the

female Leidynema appendiculata has a cylindrical anterior esophagus; presence of intestinal diverticulum; posterior intestine with a single loop; and vulva located near middle of body.

The H. diesingi male has an anterior esophageal pseudobulb; tail obliquely truncate with a long tapering thickened portion attached dorsally. The L. appendiculata male has a prebulbular swelling in the esophagus and truncate tail with very small dorsally attached end piece. The distinction between the males of these species is no doubt correct for on several occasions copulation was observed in both H. diesingi and L. appendiculata. While the primary object of this report was to make a study of L. appendiculata, it was found convenient to make comparisons with but not illustrations of H. diesingi.

Females of L. appendiculata (Fig. 2) vary from 1.9 to 4.0 mm. in length and from 0.13 to 0.35 mm. in width. They have a comparatively stout body with a long tapering tail. The integument is closely annulated from the anterior end to the tail. Lateral alae extend from the region of the esophageal bulb posteriorly, terminating in spine-like projections. The mouth as shown by Chitwood is surrounded by eight large submediam labio papillae. The esophagus consists

of a long cylindrical anterior portion which connects posteriorly by a narrow isthmus with the esophageal bulb. From the anterior part of the intestine a large diverticulum, varying considerably in size, emerges laterally and posteriorly. In the posterior half of the worm the intestine forms a distinct loop. The nerve ring is usually located in the region of the prebulbular swelling. The excretory pore is posterior to the esophageal bulb.

The essential parts of the female reproductive organs are illustrated in figures 2 and 3. The vulva of L. appendiculata is located slightly posterior to the middle of the body. From the genital orifice the vagina extends dorsally to the opposite side of the body where it joins the opposed uteri. The uteri are capable of great distention, hence their length and size vary with the number of eggs contained and the age of the worm. Ordinarily the anterior uterine branch extends forward to the distal end of the intestinal diverticulum and the posterior branch to slightly beyond the intestinal loop. The oviducts, leading from the uteri, extend for varying distances in the same direction as their respective uteri, then each duct reverts and follows an irregular course to the opposite end of the body cavity. From there the ducts make another complete turn and each branch then continues its new course for a

distance which varies in different specimens, until it unites with its respective ovary. The anterior ovary extends from the region of the intestinal diverticulum to the posterior third of the body while the other ovary extends from the extreme posterior end of the body to the anterior third of the worm. The shell glands appear to be located in the distal portions of the uteri.

The range in body lengths of the mature males (Fig. 1) of L. appendiculata is 0.8 to 0.9 mm. and the range in width is from 0.05 to 0.065 mm. The integument is much like that of the female. The tail is short with a pair of large preanal, a pair of small postanal and a pair of indistinct subdorsal papillae, also a very minute pointed dorsal end piece.

The digestive system is composed of an esophagus which closely resembles that of the female. The intestine is simple without a diverticulum or loop. In the posterior half of the body is the reproductive system. The testis is a compact structure in the middle of the worm. It opens directly into the sperm duct which appears to be surrounded throughout by large glandular cells. A short ejaculatory duct opens into the cloaca. A single spicule is present.

## EXPERIMENTAL DATA AND OBSERVATIONS

### Securing Nematode-Free Cockroaches

For most of the experiments it was necessary to use nematode-free cockroaches. To secure such roaches three methods were employed.

1. By rearing cockroaches from oöthecae as described. Records kept of the growth of these insects coincide closely with the reports by Haber and Marlatt (1928). This method was the most satisfactory except for the time involved before the roaches were large enough for experimental work.

2. After the nematode eggs have been discharged with the fecal matter of the roach a minimum of two days is required before they have developed to the infective stage. Taking advantage of this roaches were transferred to a clean habitat every day and in that way reinfestation was prevented. In due time depending on the longevity of their parasites, these roaches became uninfested. Some of these insects were used for experiments while daily transfers were made but the data secured is of doubtful value for in making transfers it is possible for the roaches to carry fecal matter containing eggs into the new containers.

3. Attempts were made to administer anthelminthics to the insects. In an experiment seven roaches which gave

positive fecal tests for worms were selected. Three of these were given sweetened water and strychnine and four were given a solution of egg albumin with thymol and sugar, all other foods being excluded. All subsequent fecal tests and examinations for nematode parasites were positive. In another similar experiment fresh rattlesnake venom was introduced into the drinking water. While this was not expected to have any effect on the hosts it apparently did not harm the parasites, either.

Oxygen was used by Cleveland (1925) to defaunate termites and other animals. He was able to kill protozoan parasites in insects in several hours without injury to the hosts when they were oxygenated at three and one-half atmospheres. At lower atmospheres a longer time was required.

In an experiment five parasitic nematodes which were placed on moist slides and subjected to one atmosphere of oxygen were dead after 48 hours of exposure, but the controls likewise died. In another experiment under the same conditions 10 hours of oxygenation greatly reduced the activity of the nematodes and oxygenation for 24 hours killed all of the worms so exposed. Of the controls some survived.

The results of these experiments are by no means conclusive, but it was assumed that if one atmosphere of

oxygen applied directly had little effect on the worms the gas under greater pressure would probably fail to affect the parasites in the intestines of their hosts. However, it is hoped that further experiments may be conducted on oxygen and other gases as anthelminthics.

## Culturing Nematode Eggs

Eggs from many species of parasitic nematodes, such as hookworms and ascarids, hatch in artificial media. The eggs of L. appendiculata and H. diesingi (Tables 3 and 4) presented a different problem as the larvae failed to emerge from their shells regardless of the media in which they were cultured. Results of five series of experiments, A, B, C, D and E, are shown in Table 3. Eggs in varying stages of development of either or both species of nematodes were used in each culture. The media consisted of compounds or combinations of materials as shown in Table 3. The nematodes lived longer in diluted than in ordinary Locke's solution. The filtered fecal extracts were made from three or four grams of cockroach fecal matter in 100 cc. of diluted Locke's solution. During the course of the experiments the temperature varied from 68° to 82° F. The cultures were maintained from four to 60 days but in no cases did the larvae develop beyond the resting embryonated

Table 3. Showing Results of Culturing Eggs in Various Media.

| Series | Species | Initial development | Culture media | Temperature | Days incubated | Maximum development |
|---|---|---|---|---|---|---|
| A | *H. diesingi* | Morula | Dilute formalin | Room | 4 | No change |
| B 1 | *H. diesingi* | Undeveloped | Distilled water | Room | 9 | Resting embryonated stage* |
| B 2 | *L. appendiculata* | Undeveloped | 0.9% saline | Room | 9 | Resting embryonated stage |
| C 1 | *L. appendiculata* | Undeveloped | Fecal matter and distilled water | Room | 6 | Resting embryonated stage |
| C 2 | *L. appendiculata* | Undeveloped | ½% peptone in saline | Room | 6 | Resting embryonated stage |
| C 3 | Mixed | Undeveloped | ½% peptone and fecal extract | Room | 6 | Resting embryonated stage |
| C 4 | Mixed | Undeveloped | Water plus drop of Meyer's egg albumin | Room | 6 | Resting embryonated stage |
| C 5 | Mixed | Morula | Water plus thymol | Room | 6 | Resting embryonated stage |
| D 1 | *L. appendiculata* | Undeveloped | Locke's solution | Room | 5 | Resting embryonated stage |
| D 2 | *L. appendiculata* | Undeveloped | Locke's solution | Room | 5 | Resting embryonated stage |
| D 3 | *L. appendiculata* | Morula | Moist fecal matter | Room | 59 | Resting embryonated stage |
| D 4 | Mixed | Undeveloped | Fecal matter and water | 98° F. | 45 | Resting embryonated stage |
| D 5 | *H. diesingi* | Eggs in dead worm | Distilled water | 98° F. | 34 | Resting embryonated stage |
| D 6 | *L. appendiculata* | Eggs in uterus | Distilled water | 98° F. | 34 | Resting embryonated stage |
| E 1 | *L. appendiculata* | Undeveloped | Locke's solution | 98° F. | 5 | Resting embryonated stage |
| E 2 | *L. appendiculata* | Undeveloped | Locke's solution | 98° F. | 60 | Resting embryonated stage |
| E 3 | Mixed | Undeveloped | Moist fecal matter | 98° F. | 38 | Resting embryonated stage |
| E 4 | *L. appendiculata* | Resting stage | Locke's solution | 98° F. | 59 | Resting embryonated stage |

*The term embryonated refers to advanced stages in development.

stage. Most of the embryos developed to this stage in four to seven days. Fecal matter collected from the natural habitats of cockroaches showed all live eggs developed to the resting embryonated stage.

An experiment (Table 4) much like the preceding one was conducted to determine the effects of different intestinal extracts on these nematode eggs. Ingredients used in making up the media were: dilute aqueous fecal extracts, a digestive fluid composed of 0.5 per cent pepsin and 0.20 per cent hydrochloric acid in water, and concentrated filtered extracts from the foregut and from the rest of the alimentary canal. The first extracts were added to cultures which contained eggs in various stages of development as shown in Table 4. After a short incubation period the degree of development was recorded; new ingredients were added from time to time and the cultures were frequently examined, but no eggs developed beyond the embryonated stage. Other experiments similar to these were conducted but discontinued due to moulds which infected and destroyed the cultures.

## Viability of the Eggs

No attempt was made to conduct viability experiments but observations are here reported on the viability of the

Table 4. Results of Culturing Eggs in Intestinal and Fecal Extracts.

| Series | Species | Egg culture medium | Initial development | Days incubated | Degree of development | New culture medium added | Additional incubation | Final degree of development |
|---|---|---|---|---|---|---|---|---|
| F 1 | *L. appendiculata* and *H. diesingi* | Fecal extract | Active and resting | 2 | Resting embryonated stage | Large intestine extract | 53 days | Resting embryonated stage |
| F 2 | *H. diesingi* | Fecal extract and digestive fluid | Active stage | 2 | Resting embryonated stage | None | 53 days | Resting embryonated stage |
| F 3 | *H. diesingi* | Fecal extract and fecal pellets | Worms fresh from roach | 2 | Active embryonated stage | Small and large intestine extract | 53 days | Resting embryonated stage |
| F 4 | *L. appendiculata* | Fecal extract | Worms fresh from roach | 2 | Active embryonated stage | None | 53 days | Resting embryonated stage |
| F 5 | *L. appendiculata* and *H. diesingi* | Intestinal contents and fecal extract | Worms fresh from roach | 2 | Active embryonated stage | None | 53 days | Resting embryonated stage |
| F 6 | *H. diesingi* | Fecal extract and animal charcoal | Resting stage | 1 | Resting embryonated stage | Small intestine extract | 52 days | No change |
| F 7 | *H. diesingi* | Fecal extract digestive fluid and animal charcoal* | Resting stage | 1 | Resting embryonated stage | Few drops picric acid | 52 days | No change |
| F 8 | *L. appendiculata* | Fecal extract and digestive fluid* | Active stage | 1 | Resting stage | | 52 days | No change |
| F 9 | *L. appendiculata* and *H. diesingi* | Fecal pellets and animal charcoal | One celled | 1 | Active embryonated stage | | 52 days | Resting embryonated stage |
| F 10 | *L. appendiculata* | Fecal extract | Active stage | 0 | Resting embryonated stage | | 51 days | No change |

* Coverslip was gently pressed on eggs to break shells.

eggs as determined in the course of other experiments. A large per cent of the cultured eggs exposed in strong indirect sunlight did not continue to develop. Light from a powerful 400 watt projection lamp, although passed through a water bath, was detrimental to most of the embryos in the early stages of cleavage, if the exposure was longer than 15 minutes. The heat from this lamp which was 40° C. may have been a contributing factor in destroying the eggs. However, observations show that the eggs of these nematodes are much more susceptible to light than ascarid eggs. Caldwell and Caldwell (1928) and Otto (1929) reported that sunlight was an important factor in the destruction of ascarid eggs. Sasaki (1928) found that short rays of light were detrimental to ova of parasitic worms. The experiments by Nolf (1932) show that wave lengths of light, ranging from 180 to 315 microns were highly fatal to ascarid ova.

In the present experiments, eggs reared under cover slips showed arrested development but continued to develop when exposed to air. Eggs retained in the bodies of dead nematodes did not divide until decomposition of the worms took place. In this instance bacteria in the decomposing worms had no visible effect on the normal eggs. Embryos in the last stages of growth can withstand considerable desiccation while those in the early stages are readily destroyed.

The eggs in moist fecal pellets of the roaches no doubt retain their viability for a considerable period as cultures (two months old) in diluted Locke's solution contained viable eggs.

Under optimum conditions development of the eggs is very rapid. The undeveloped eggs often reach the two cell stage (Plate II) in less than an hour and 12 hours later development has progressed to the stage illustrated by figure 16. Eggs containing active stage embryos (Fig. 18) were found 24 hours after they were laid. The active stage usually does not last longer than two or three days. Three to seven days are required for the embryo to develop to the infective, or resting embryonated stage (Fig. 20). Cold and heat retard or influence the rate of development. In the winter months development of the embryos may take weeks while at higher temperatures the infective stage is reached in a few days. No attempt was made to find the optimum temperature but excellent growth took place at 98° F.

## Transmission

It was assumed by Bütschli (1871) that transmission of these nematodes is direct. To the writer's knowledge no previous transmission experiments have been made with parasitic nematodes from the family Blattidae. A series of

experiments was carried out to determine the stage at which the eggs became infective and the means of transmission. The results of these experiments are given in Table 5. These transmission experiments were facilitated by starving the cockroaches for a week or two prior to the time of feeding. The nematode eggs were incubated to the desired stage and then fed to the cockroaches with food or water.

The roaches of group A were fed L. appendiculata eggs which were in various stages of development. After four days one was killed and examined; it was infested. The others were killed on the fifth and sixth days respectively and both were without worms. In group B four cockroaches were given a mixed culture of eggs in the resting embryonated stage; on examination three of them were infested.

Group C was given one culture of L. appendiculata eggs in the active embryonated stage and seven days later a second culture of eggs at the same stage. Six of the roaches of the last mentioned group were negative and one was positive. In group D eggs of the resting embryonated stage were fed to three roaches and two of them were positive in 25 days. H. diesingi eggs in an active embryonated stage were fed to the roaches of group E; in from six to seven days all were negative. The four cockroaches of group F took eggs of H. diesingi in the resting embryonated

Table 5. Showing Results of Transmission Experiments.

| Cockroaches: Group | Roach: No. | Nematodes: Species | Egg culture medium | Degree of development of eggs fed | Days parasitism before examination | Worms recovered |
|---|---|---|---|---|---|---|
| A | 1 | L. appendiculata | Fecal extract | Many stages | 4 | 6+ |
| | 2 | L. appendiculata | Fecal extract | Many stages | 5 | 0 |
| | 3 | L. appendiculata | Fecal extract | Many stages | 6 | 0 |
| B | 1 | L. appendiculata and H. diesingi | Fecal matter from infested roaches | Resting embryonated stage | 3 | 1+ |
| | 2 | L. appendiculata and H. diesingi | Fecal matter from infested roaches | Resting embryonated stage | 4 | 4+ |
| | 3 | L. appendiculata and H. diesingi | Fecal matter from infested roaches | Resting embryonated stage | 6 | 0 |
| | 4 | L. appendiculata and H. diesingi | Fecal matter from infested roaches | Resting embryonated stage | 7 | 4+ |
| C | 1 | L. appendiculata | Diluted Locke's solution | Active embryonated stage | 5 | 0 |
| | 2 | L. appendiculata | Diluted Locke's solution | Active embryonated stage | 7 | 0 |
| | 3 | L. appendiculata | Diluted Locke's solution | Active embryonated stage* | 21 | 0 |
| | 4 | L. appendiculata | Diluted Locke's solution | Active embryonated stage* | 21 | 0 |
| | 5 | L. appendiculata | Diluted Locke's solution | Active embryonated stage* | 21 | 0 |
| | 6 | L. appendiculata | Diluted Locke's solution | Active embryonated stage* | 24 | 0 |
| | 7 | L. appendiculata | Diluted Locke's solution | Active embryonated stage* | 24 | 1+ |
| D | 1 | L. appendiculata | Diluted Locke's solution | Resting embryonated stage | 25 | 0 |
| | 2 | L. appendiculata | Diluted Locke's solution | Resting embryonated stage | 25 | 1+ |
| | 3 | L. appendiculata | Diluted Locke's solution | Resting embryonated stage | 25 | 5+ |
| E | 1 | H. diesingi | Diluted Locke's solution | Active embryonated stage | 6 | 0 |
| | 2 | H. diesingi | Diluted Locke's solution | Active embryonated stage | 6 | 0 |
| | 3 | H. diesingi | Diluted Locke's solution | Active embryonated stage | 6 | 0 |
| | 4 | H. diesingi | Diluted Locke's solution | Active embryonated stage | 6 | 0 |
| | 5 | H. diesingi | Diluted Locke's solution | Active embryonated stage | 6 | 0 |
| | 6 | H. diesingi | Diluted Locke's solution | Active embryonated stage | 7 | 0 |
| F | 1 | H. diesingi | Diluted Locke's solution | Resting embryonated stage Two months old culture | 5 | 7+ |
| | 2 | H. diesingi | Diluted Locke's solution | Resting embryonated stage Two months old culture | 6 | 4+ |
| | 3 | H. diesingi | Diluted Locke's solution | Resting embryonated stage Two months old culture | 6 | 0 |
| | 4 | H. diesingi | Diluted Locke's solution | Resting embryonated stage Two months old culture | 6 | 0 |
| G | 1 | L. appendiculata | Diluted Locke's solution | Resting embryonated stage | 2 | 0 |
| | 2 | L. appendiculata | Diluted Locke's solution | Resting embryonated stage | 2 | 0 |
| | 3 | L. appendiculata | Diluted Locke's solution | Resting embryonated stage | 4 | 0 |
| | 4 | L. appendiculata | Diluted Locke's solution | Resting embryonated stage | 7 | 0 |
| | 5 | L. appendiculata | Diluted Locke's solution | Resting embryonated stage | 7 | 0 |
| | 6 | L. appendiculata | Diluted Locke's solution | Resting embryonated stage | 7 | 0 |
| | 7 | L. appendiculata | Diluted Locke's solution | Resting embryonated stage | 9 | 0 |
| | 8 | L. appendiculata | Diluted Locke's solution | Resting embryonated stage | 9 | 0 |

*A second culture of active embryonated eggs was fed seven days later.

stage from a two month old culture; two of them were positive in five to six days. The last group (G) was composed of eight hosts which were given a week old culture of L. appendiculata eggs. All of the roaches used in the last transmission experiment were negative in from two to nine days. Although these eight insects were very much younger than those used in the other experiments, mature nematodes have been removed from specimens of corresponding size. Of the total of 35 cockroaches used in these experiments nine were positive. Seventeen roaches fed eggs in the resting embryonated stage yielded eight positive cases. On the other hand, transmission in the active embryonated stage was successful in only one case out of 13.

In these experiments a limited number of eggs was fed to the cockroaches, consequently, the infestations were never very heavy. Repeated feedings of large numbers of eggs probably are necessary to secure a higher incidence and heavier infestations. Under natural conditions the roaches constantly feed on infected food or fecal matter and thus probably reinfest themselves. While only 25.7 per cent of the cockroaches fed embryonated eggs of the worms became infested the results of the experiments show conclusively that transmission is direct. The results also show that eggs in the resting embryonated stage are infective but in-

dicate that those in the active embryonated stage are seldom infective.

## LIFE CYCLE OF THE NEMATODES

### Process of Egg Laying

Due to the transparency of the cuticular and muscular layers of the living nematodes it was possible to determine much of the anatomy of the reproductive organs without special treatment. The general organization of the reproductive system is much like that found in most nematodes. The germinal zone containing nuclei is located in the distal portion of the ovary. The nuclei migrate away from the distal end where ova are formed. In the developmental area the ova are arranged in layers much like piles of discs. As the ova approach the proximal end they become surrounded with yolk material. The eggs pass from the ovaries into the oviducts where they appear as irregular masses but midway between the ovary and uterus they assume a more characteristic shape. The shell glands appear to be near the regions where the oviducts lead into the uteri. Just where fertilization takes place was not determined. Eggs in the uteri are usually unsegmented; however, in a few living specimens intra-uterine eggs were developed slightly beyong the two-cell stage. In culture media the gravid females often

underwent a period of rapid egg laying until the uteri were empty while other females retained their eggs several days. Eggs may be discharged as rapidly as one every five seconds. In one instance a worm laid 60 eggs in 90 minutes. The eggs are oval, elongate and flattened on one side as shown in Plate II. They are thin shelled and in different specimens vary considerably in length and width. Magalhaes (1900) states that the shell of Thelastoma bulhoesi (Magalhaes, 1900) Travassos, a related species, is composed of two parts of which the internal one is very thin and becomes visible after the use of reagents.

## Development of the Egg

At the time of extrusion the eggs are undeveloped or in the very earliest stages of cleavage. During the early cleavage stages (Figs. 4 to 10) the reorganization of the protoplasm is accompanied by Brownian movement. This constant activity of the dark and light protoplasmic granules is evidence of rapid internal physico-chemical activity. In the undeveloped egg (Fig. 4) the granular protoplasm is distributed throughout the egg. The protoplasm undergoes irregular shrinkage (Figs. 6 and 6) away from the shell. These variable contractions of the cell continue until it arrives at a stage (Fig. 7) which, due to the arrangement

of protoplasm has the external appearance of a morula. In the immediately succeeding stages the protoplasm from one side of the cell is thrust out in amoeboid fashion until it is slightly larger than illustrated (Fig. 8). This structure which appears to be a polar body does not separate from the cell proper but appears to become ingulfed by the elongating cell (Fig. 9). About the time when the union between the cell proper and the polar body is completed a constriction appears in the middle of the elongated cell (Fig. 10). As shown in Figure 11, cleavage is unequal. The other stages (Figs. 12 to 15 represent typical four, eight, sixteen and thirty-two cell stages. During the stage which may be a morula (Fig. 16) there is no obvious external change.

After this stage a period of embryonic growth and development ensues. The embryo (Fig. 17) has a flattened anterior end and a tail bud at the posterior extremity. The structure of the cells is no longer obvious without staining. Growth takes place both anteriorly and posteriorly until a motile tadpole-like embryo (Fig. 18, the active stage) is formed. The active stage embryo has a small pointed tail and a comparatively large blunt anterior end. The digestive tract consists of a simple undifferentiated tube. This young form contracts its body and squirms about but it

retains its same relative position within the egg. As the development of the embryo progresses to the next stage (Fig. 19) the wriggling decreases and then ceases. The young form is contracted and non-motile, the tail being reduced to a mere stub. The anterior esophagus and esophageal bulb are visible.

The last stage (resting embryonated) before the embryo hatches resembles the one just described, but shows a much higher degree of development. The embryo is more contracted and rounded. There is evidence of a striated cuticle. The characteristic esophageal bulb can be seen distinctly in the posterior half of the body. The anterior esophagus is sufficiently well developed to be recognized. Due to the opacity of the specimens the structure in the posterior region could not be studied.

## Hatching of Eggs

Since the natural mode of transmission of these nematodes is through ingestion of infective eggs, hatching must take place somewhere in the alimentary canal of their host. In order to determine where hatching occurs the crop, midgut and hindgut of each cockroach examined were searched separately for eggs and larvae. In a few instances eggs with mature embryos were found in the crop but never in the

midgut. The hindgut often contained empty egg shells and eggs but the latter were always in the early stages of development. Young larvae were present in the posterior part of the midgut and in the hindgut but absent in the rest of the digestive tract.

The passage of the eggs through the gut of the roach may be quite rapid. Some of these insects were given food colored with carmine and in several hours the color was found in the midgut and in less than eight hours the contents of the hindgut, too, were stained. No doubt the rate at which the food passes down the gut of the roach is influenced by such factors as activity of the insect, temperature and so forth. From the tests made it appears that the larvae hatch in the posterior part of the midgut.

## Growth and Development of the Larvae

Some of the developmental stages of L. appendiculata are illustrated in Figures 21 to 24. As the time of hatching was not determined it is impossible to give the exact age of the embryos. The illustrations merely show some of the important anatomical changes in different stages of growth.

The youngest larvae found measured about 150 to 170 microns in length. At this stage it was impossible to dis-

tinguish L. appendiculata from H. diesingi. In a slightly older larva (Fig. 21) which measured 208 microns in length the esophagus resembles that of a mature L. appendiculata; the intestine is a simple tube. At the corresponding stage the larvae of H. diesingi have an esophageal pseudobulb anterior to the true bulb. The entire cuticle of both larval forms is annulated much like the adults.

A larva 430 microns long (Fig. 22) was recovered 14 days after infestation. In this specimen the esophagus is divided into distinct anterior and posterior parts connected by a narrow isthmus (midpiece). The intestinal walls were irregular and the anterior part had begun to enlarge. The larvae illustrated in Figure 23, although over a millimeter long, were but slightly more developed than the preceding worm. There is an enlarged cardia and the intestinal diverticulum appears to develop as a pouch from the anterior intestine. Many of the larvae, as the two just described, also show great variations in the length of the tail.

Specimens of L. appendiculata larvae with represent stages in the development between those shown in Figures 23 and 24 are so opaque that in spite of treatment the internal anatomy could not be clearly determined. The opacity may be due to the two-layered cuticula prior to moulting. All of these young sausage-like forms are comparatively much

shorter and thicker than the other larvae. However, in these specimens the intestine appears thick walled, irregular and slightly coiled. The esophagus is typical and the genital primordium a small mass of heavily stained cells.

The immature worm at the next stage (Fig. 24) bears much resemblance to the adults. It is characterized by a typical esophagus, a conspicuous intestinal diverticulum and a greatly coiled posterior intestine. The genital primordium, a small protuberance extending dorsally from the genital orifice, is located in the region of the diverticulum. A slightly later stage (Fig. 25) shows a little modification in most of the essential structures. The body is somewhat longer and the posterior intestine more distended and less convoluted; the most marked change is in the reproductive organs. Because of the parasite's growth the genital primordium is posterior to the intestinal diverticulum. The reproductive anlage extends from the vulva dorsally to the opposite side of the body where it forks into short branches, one anterior, the other posterior. The worms illustrated in Figures 24, 25 and 26 measure respectively, 1.2, 1.3, and 2 mm. in length.

The immature female (Fig. 26) is much the same as the two forms last described but the anatomical positions of its internal organs do not differ significantly from the adult

worms. In most but not in all of the older immature and fully developed worms the esophagus is reduced in comparative length and size. The intestine of this young worm has a single loop in the anterior part of the body. The reproductive organs consist of a vulva, a short vagina, an immature anterior and posterior uterus. Each of these uteri is joined with an undeveloped ovary. At this stage the oviducts cannot be distinguished from the uteri. In the mature worm (Fig. 2) the organs of the reproductive system are larger and distributed throughout the body cavity posterior to the esophagus.

A study of the anatomy of the young and adult L. appendiculata revealed that there is no correlation between the larvae of the same stage in development (Figs. 23 and 24) or adults and the position and size of the parasites' organs. Contrary to the statements made by Bütschli the length of the worm's tail is not a criterion of age for the immature forms (Figs. 22 and 23) show great variation in the length of the tail. The same statement applies to the length of the esophagus, location of the nerve ring and the location of the reproductive organs. This condition makes measurements such as the Cobb formula valuable only when type species are described. On the contrary the external organs such as the vulva, anus and alae

are found in the corresponding location in all of the specimens which show the same degree of development.

## SUMMARY

1. Of the Periplaneta americana collected at Manhattan, Kansas, 85 per cent were infested with nematodes. From this group, 94 per cent of the immature, 87 per cent of the adult females and 79 per cent of the mature male cockroaches were infested with parasitic worms. Two species of Oxyurids, Leidynema appendiculata and Hammerschmidtiella diesingi, inhabit the intestinal tract of the cockroach and of the two species the first was more numerous. The size of infestation in each roach was highest in the females and lowest in the young P. americana. The infestations ranged from one to 21 mature nematodes per roach.

2. Although the females of L. appendiculata were very numerous, males were not found in most of the hosts. Males of H. diesingi were almost as numerous as the females of the same species.

3. A study of the anatomy of L. appendiculata was made with special emphasis on the reproductive system of the female.

4. Uninfested cockroaches were obtained by rearing the young from oöthecae.

5. Eggs of both species can survive more desiccation in the later than in the early stages of development. In a moist chamber eggs remained viable for at least two months. Eggs of all stages when exposed to direct strong artificial or natural light for 15 minutes failed to develop.

6. Regardless of the media employed eggs failed to hatch *in vitro*.

7. Feeding experiments proved that transmission is direct. Eggs in the resting embryonated stage are infective; those in the active embryonated stage do not appear to be infective.

8. The process of egg laying in *L. appendiculata* was observed and different phases in the development of the eggs recorded.

9. Fertilized eggs incubated at 37° C. in dilute Locke's solution develop to the active embryonated stage in 20 to 36 hours; in four to seven days they develop to the resting embryonated stage.

10. Hatching takes place in the posterior region of the midgut and subsequent development in the hindgut. Characteristic stages in the development and growth of the larvae were studied.

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The outlines for the drawings were made with the aid of a microprojection apparatus.

## ABBREVIATIONS

| | | | |
|---|---|---|---|
| a.c | - anterior esophagus | o' | - anterior ovary |
| an | - anus | o'' | - posterior ovary |
| ann | - annulations | ov' | - anterior oviduct |
| ant.r | - anterior branch reproductive system | ov'' | - posterior oviduct |
| ca | - cardia | po.an.p | - postanal papilla |
| cl | - cloaca | pos.r | - posterior branch reproductive system |
| e | - esophagus | | |
| e.b | - esophageal bulb | pr.an.p | - preanal papilla |
| eg | - egg | r | - rectum |
| ej.d | - ejaculatory duct | s.d | - sperm duct |
| e.p. | - end piece | sd.p | - subdorsal papilla |
| e.ps | - esophageal pseudobulb | sp | - spicule |
| g.c | - glandular cells | spr | - spermatozoa |
| g.p. | - genital primordium | ta | - tail |
| int | - intestine | te | - testis |
| int.d | - intestinal diverticulum | ut' | - anterior uterus |
| m | - mouth | ut'' | - posterior uterus |
| m.p | - midpiece | va | - vagina |
| n.r | - nerve ring | vu | - vulva |

# EXPLANATION OF PLATES

PLATE I

Fig. 1. Mature Leidynema appendiculata male.

Fig. 2. Mature L. appendiculata female.

Fig. 3. Diagrammatic drawing showing reproductive system of female L. appendiculata.

# PLATE I.

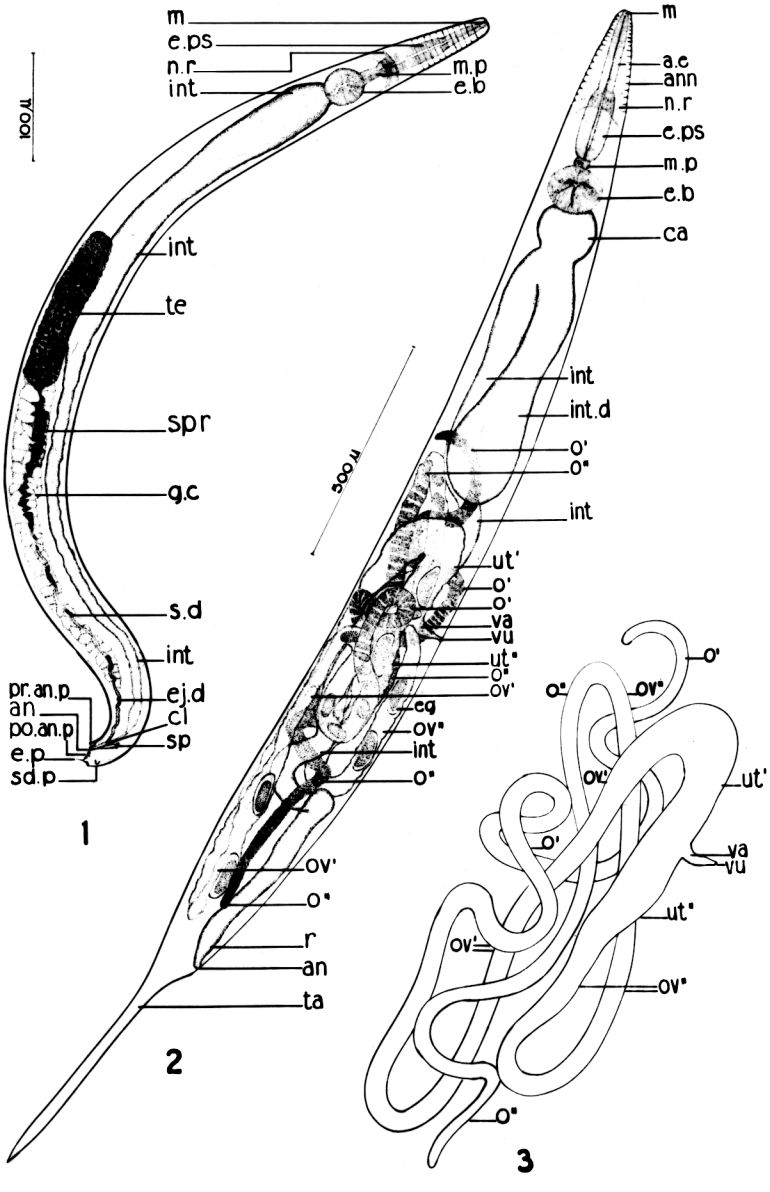
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PLATE II.

Figs. 4-20. *Leidynema appendiculata* eggs.

4. Fertile egg.

5- 6. Protoplasmic shrinkage from egg shell.

7. One cell stage.

8. Polar body formation.

9. Polar body merging with cell proper.

10. Beginning of cleavage.

11-15. Cleavage stages.

16. Morula.

17. Pre-active embryonic stage.

18. Active embryonic stage.

19. Pre-resting embryonic stage.

20. Resting embryonic stage.

# PLATE II.

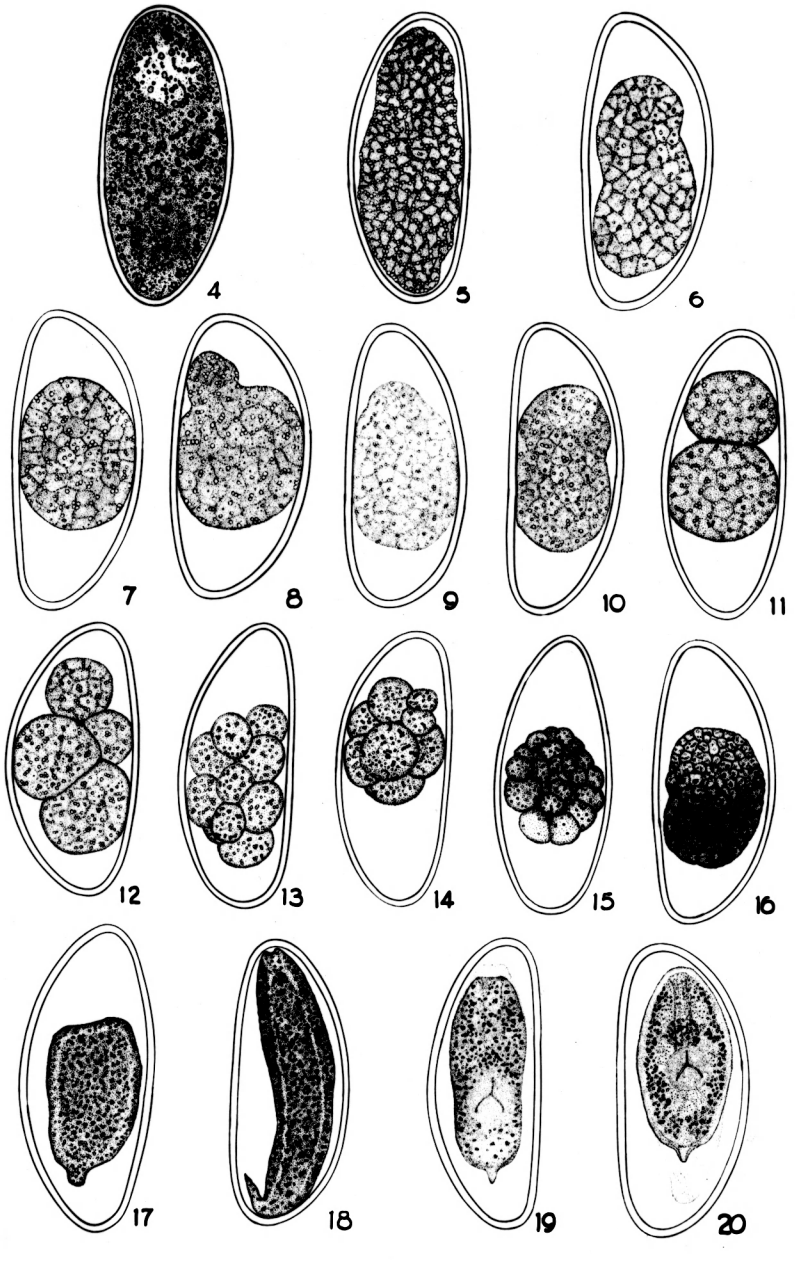


PLATE III.

Figs. 21-26. Immature Leidynema appendiculata.

21. First stage larva.

22. Ten-day old female.

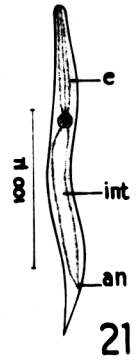
23. Young female showing enlargement of cardia.

24. Female showing intestinal diverticulum, coiled intestine and genital primordium.

25. Female showing a stage in development of the reproductive system.

26. Young female with immature reproductive system.

# PLATE III.

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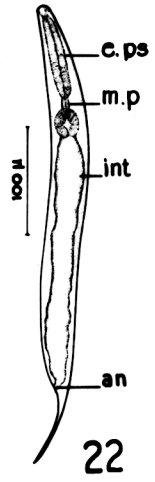


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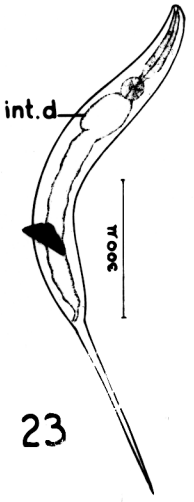


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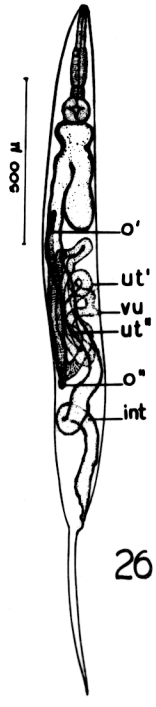


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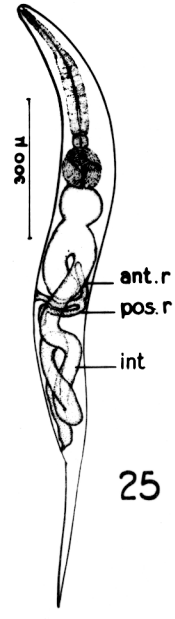


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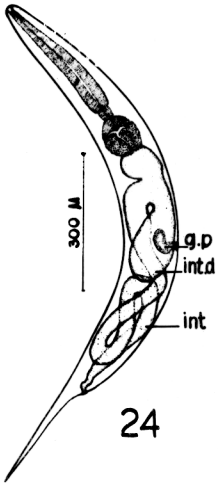


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