

Life History of *Microtetrameres centuri* Barus, 1966 (Nematoda: Tetrameridae) I. Juveniles

CHARLES J. ELLIS¹

Abstract: Anatomical details of experimentally reared juveniles of *Microtetrameres centuri* are reported. These details are compared to results of similar studies of other *Microtetrameres* species.

Little information concerning anatomical characteristics of juvenile *Microtetrameres* spp. exists. Seurat (12, 13, 14), one of few investigators to describe *Microtetrameres* juveniles, included the following features in his descriptions of third-stage and what he termed fourth-stage juveniles of *Tropidocerca* (= *Microtetrameres*) *spiralis* recovered from naturally infected hosts: length of tail, distance from head to nerve ring and to excretory pore, body length and width, length of buccal capsule, lengths of the two pharyngeal portions as well as the ratio of the entire pharynx to the body length. Schell (11) and Cram (5) have also reported on juveniles stages of *Microtetrameres* spp.

This study was initiated after finding that 53.7% of the 173 meadowlarks (*Sturnella neglecta* and *S. magna*) (7) collected in Iowa were infected with *Microtetrameres* spp. The high percentage of infection, the wide (115 species) host range of *Microtetrameres*, and the stomach-content study indicated that meadowlarks were suitable hosts for this nematode.

METHODS AND MATERIALS

Female *Microtetrameres centuri* gravid with embryonated eggs were fed to grasshopper nymphs (*Melanoplus* spp.). The nymphs were examined for juvenile nematodes at various post-feeding times. The

living juveniles were examined under phase-contrast and dark-field microscopy, fixed by various methods and mounted in either glycerine jelly or CMC-10 (a General Biological Supply House mountant). Living and fixed juveniles were measured using a calibrated ocular micrometer or microprojection techniques. All drawings, except those noted, were made with the aid of a microprojector.

RESULTS

EGG: Embryonated eggs were oval, slightly flattened on one surface (Fig. 1) with a boss on at least one end. Unembryonated eggs were collapsed and crescent-shaped in cross-section. The egg shell averaged 2 to 3 μ in thickness. Filaments were lacking. Cephalic projections of the juvenile (Figs. 2, 4, 5) sometimes were seen while the juvenile was still within the shell.

Measurements of 10 eggs from female *M. centuri* raised experimentally in a pigeon averaged 50.7 μ long by 35.3 μ wide. Similar measurements of 82 eggs of female *M. centuri* recovered from naturally infected meadowlarks averaged 50.5 μ long by 35.2 μ wide.

FIRST-STAGE JUVENILES: Juveniles emerged within a few hours after eggs were ingested by a grasshopper nymph. Some juveniles recovered from the grasshopper's fore-gut were seen partially emerged (Figs. 2, 11). First-stage juveniles escaped from the egg with either end emerging first. Both methods have been reported for *M. inermis* (12). In 13 eggs nine juveniles had emerged anterior first (Figs. 2, 11) and four juveniles

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tail first. In one instance, a juvenile had broken the shell but both of its ends were still within the egg shell. Thirty minutes later this juvenile had escaped tail first.

First-stage juveniles possessed blunt anterior (Figs. 3, 11) and pointed tails (Fig. 3). The anterior end of a living first-stage juvenile changed from convex to concave as the animal moved (Figs. 4, 5). In this aspect, *M. centuri* first-stage juveniles resembled juveniles of what appeared to be *Tetrameres*, as illustrated by Lieberkuhn (9). A group of three minute refractile cephalic projections (Figs. 4, 5) appeared anteriorly. These projections do not resemble cephalic hooks (10). The position of this group of projections was modified somewhat in accordance with the movement of the anterior region of its body. The rapid movement of living juveniles and the minute size of these projections made observations difficult. Movement, together with the action of the cephalic projections, may have added measurably in the migration of juveniles within the tissue of their intermediate host. These projections were similar to the "aiguillon cephalique" mentioned by Chabaud (2) who considered them aids in permitting first-stage juveniles to escape from their eggs and to penetrate the intermediate host's gut wall.

The resemblance between a first-stage juvenile of *M. centuri* and a microfilaria is remarkable. About 50 μ from the anterior end of a living first-stage juvenile a clear area similar to the excretory pore of a microfilaria was seen. A spot resembling the anal pore of a microfilaria was seen approximately 50 μ from the tail. Between these two areas but closer to the posterior one was a structure resembling a short length of intestine. Presumably the intestine was connected to the anal pore, but it did not appear to extend the length of the juvenile. Within the "lumen" of this structure were three or

four refractile bodies which moved slightly as the nematode moved. Nuclei were seen throughout the living body.

At the anterior end of some living specimens the area destined to become the pharynx in older juveniles extended posteriorly about 20 μ . Granules were visible within this region in some living individuals. As the nematode moved, the granules moved rhythmically, as did the three or four refractile bodies near the anal pore. No connection between the anal pore and the anterior region was seen under bright-field or phase-contrast microscopy. Because of their synchronized movements, however, the anterior and posterior groups of refractile bodies appeared to be related.

Early first-stage juveniles were distinguished from late first-stage juveniles by their size and cuticular striations. Two types of striae were noted on first-stage juveniles. Discontinuous striations appearing as rings of tiny dots instead of continuous lines (Figs. 2, 3, 4, 5) occurred only along the anterior 16 to 20 μ of the body of the early first-stage juveniles. A second type of striation, characteristic of older first-stage juveniles, was continuous. Such striae were located posterior to the discontinuous ones.

From a point slightly anterior to the posterior discontinuous striation, two slender lines, one on either side of the body, extended nearly to the posterior end of the juvenile (Fig. 2). These were seen in numerous living and fixed specimens and probably were lateral chords.

Living juveniles displaying characteristics of both first-stage and second-stage were recovered 16 days after feeding eggs of *M. centuri* to a grasshopper nymph. These juveniles (Figs. 6, 7, 8, 9) were similar to second-stage juveniles in size and in characteristic cephalic projections. They were classified, consequently, as first-stage juveniles under-going ecdysis to the second stage.

Transverse cuticular striations were present along the length of most specimens. In others such transverse striations were limited to the anterior region. A well-developed sheath, slightly raised from the cuticle, and bearing the cephalic projections of first-stage juveniles covered the rounded anterior end of each specimen. A clear area, consisting of two separate translucent spots with definite boundaries and approximately 3–5 μ in diameter, was visible within 13 μ of the anterior end. Perhaps these spots represent glands associated with histolytic activity. The excretory pore on many of these juveniles was easily located slightly more than 80 μ from the anterior end. No evidence of a mouth was seen. The first 40 μ of the digestive tract appeared as a fine line and no lumen was visible. Posteriorly, the gut expanded, reaching its maximum width at the pharyngointestinal junction. This junction was plainly visible 130–145 μ posterior to the anterior end. The lining of the digestive tract could be traced easily under dark-field microscopy. Body musculature surrounding the tract was seen under phase-contrast microscopy. In some juveniles, foreign material was observed within the gut. The anus was apparent and measured

approximately 8 μ in diameter. It opened internally into a nearly spherical chamber (Fig. 7). Unidentified material was seen protruding from the anus of some juveniles. No genital primordium was visible under dark-field or phase-contrast microscopy.

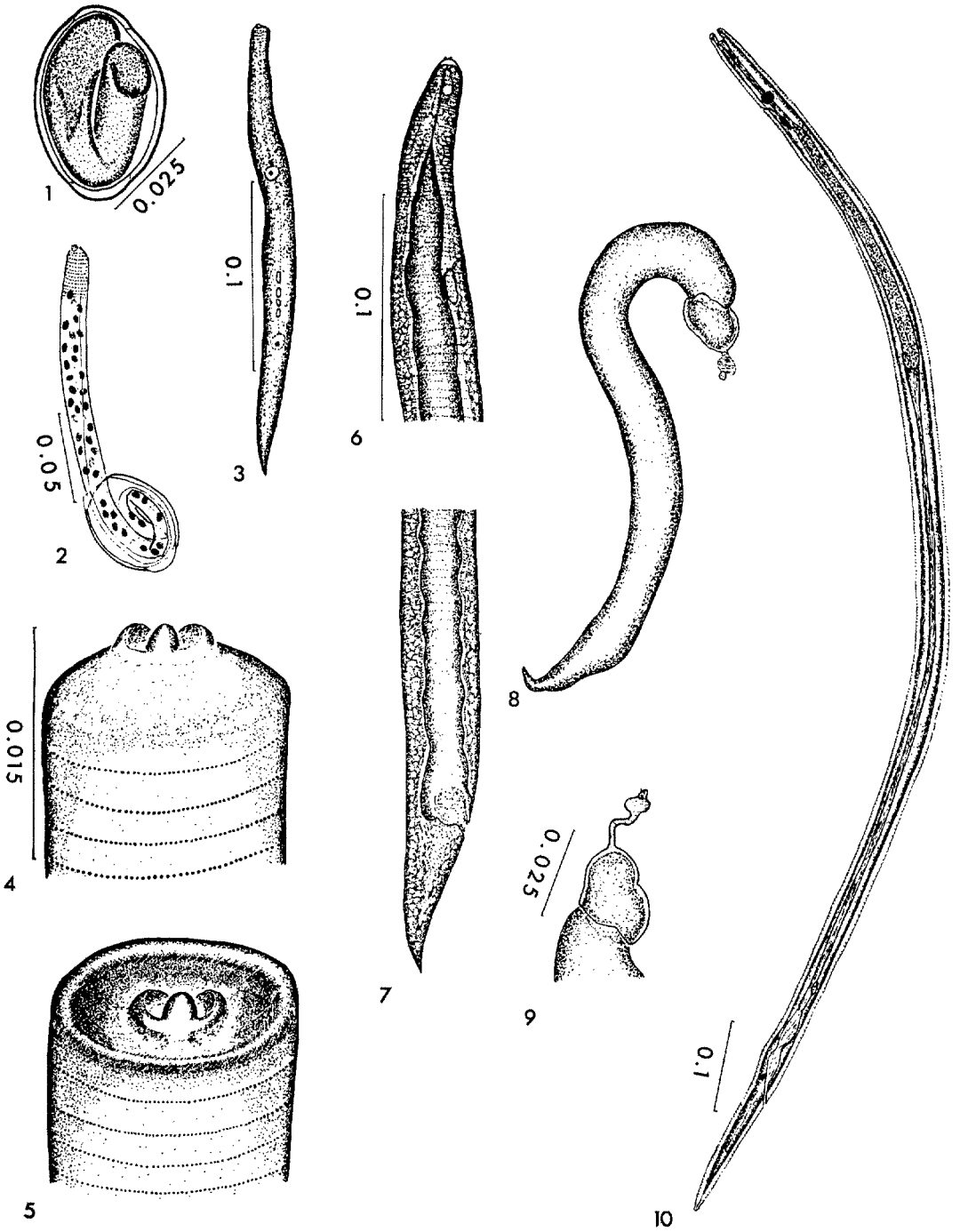
Juveniles developed from eggs fed to grasshoppers in 40 days. The following dimensions are of living and fixed first-stage juveniles:

- 2 days old (fixed) (10 specimens); length 190 μ (148–216), width 12.1 μ (10–16)
- 4 days old (living) (10 specimens); length 264 μ (235–289), width 20.8 μ (19.3–21.4)
- 5 days old (living) (10 specimens); length 318 μ (300–326), width 15 μ (11.0–21.4)
- 6 days old (living) (9 specimens); length 265 μ (245–286), width 18 μ (18)
- 8 days old (living) (8 specimens); length 336 μ (321–353), width 21.4 μ (21.4)
- 8 days old (fixed) (4 specimens); length 270 μ (257–300), width 21 μ (21)

SECOND-STAGE JUVENILES: Juveniles molted from first to second-stage between 8 and 16 days after challenge (Table 1). The length of development time appeared to

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- FIG. 1. Egg of *M. centuri* containing first-stage juvenile (in mm).
- FIG. 2. First-stage juvenile emerging from egg (in mm).
- FIG. 3. Living four day old first-stage juvenile from experimentally infected grasshopper nymph (in mm).
- FIG. 4. First-stage juvenile, anterior end extended showing cephalic projections. Free hand drawing (in mm).
- FIG. 5. First-stage juvenile, anterior end withdrawn. Same scale as Fig. 4. Free hand drawing.
- FIG. 6. Living first-stage juvenile recovered from grasshopper nymph 16 days post-feeding. Anterior end. Ecdysis commencing (in mm).
- FIG. 7. Posterior end, same specimen and same scale as Fig. 6.
- FIG. 8. Fixed first-stage juvenile undergoing ecdysis to second-stage juvenile approximately 16 days after feeding grasshopper nymph. Same scale as Fig. 6.
- FIG. 9. Anterior end, first-stage juvenile undergoing ecdysis. Specimen recovered from grasshopper nymph approximately 16 days post-feeding. Note presence of cephalic projections on sheath (in mm).
- FIG. 10. Third-stage juvenile, excysted. Specimen mounted in glycerine jelly and recovered from grasshopper nymph 38 days post-feeding (in mm).



be correlated positively with the temperature of the laboratory. Lower temperatures extended the development time.

Second-stage juveniles had convex anterior ends in contrast to first-stage juveniles with anterior ends varying from concave to convex as the living juvenile moved. Some fixed second-stage juveniles had a knob-like structure at the anterior end. This probably was the point at which the juvenile emerged from its sheath.

During the molt between first and second-stage, the cephalic projections, characteristic of first-stage juveniles, were shed with the sheath (Figs. 8, 9). The tail of a second-stage juvenile was abruptly bent (Figs. 7, 8, 13) and terminated in a sharp point lacking a "bouton." No striae were seen on early second-stage juveniles. However, they were visible on first-stage juveniles undergoing ecdysis to second-stage. Dimensions of eight second-stage living juveniles were: length 435 μ (385–514), width 36 μ (32–43). They were approximately 1.3 times as large as first-stage juveniles.

THIRD-STAGE JUVENILES: The second-stage juveniles molted to third-stage juveniles of nine days in the arthropod host (Table 1). Juveniles at this stage were infective. They were encysted within the perivisceral sinus of the intermediate host's hemocoel (Fig. 17). Usually only one juvenile occupied a cyst (Fig. 12) although as many as eight per cyst were encountered. Juveniles within these cysts moved actively and, if a cyst were punctured, the juvenile

TABLE 1. Summary of feeding experimental hosts of *Microtetrameres centuri*.

<i>Melanoplus</i> spp.	Development time	Stage recovered
2 hosts	1 day post challenge	J1
3 hosts	2 days post challenge	J1
3 hosts	3 days post challenge	J1
3 hosts	4 days post challenge	J1
2 hosts	5 days post challenge	J1
1 host	6 days post challenge	J1
1 host	7 days post challenge	J1
3 hosts	8 days post challenge	J1
1 host	8 days post challenge	J2*
1 host	15 days post challenge	J2
1 host	16 days post challenge	J2
20 hosts	25 to 68 days post challenge	J3

* Classified as J1 molting to J2.

emerged readily. This activity was observed also in *M. helix* (5).

Each third-stage juvenile possessed a complete digestive tract (Fig. 10). A tubuliform buccal capsule was apparent, but its cuticular lining was not as distinct as that of the adult (7, 8). The buccal capsule of a third-stage juvenile was nearly as long as that of an adult but only half its width. Like third-stage juveniles of *M. spiralis*, the buccal capsule of *M. centuri* is slightly expanded at its oral opening (14). Four cephalic papillae lateral to its buccal capsule were present at this stage. No cervical papillae were noted.

A well-developed pharynx was evident in living and fixed third-stage juveniles. Its lining in those specimens mounted in CMC-10 was more noticeable than in those specimens mounted in glycerine jelly. Its muscular and glandular portions were visible regardless of mountant used. However, specimens mounted in CMC-10 provided greater clarity of pharyngeal detail. The

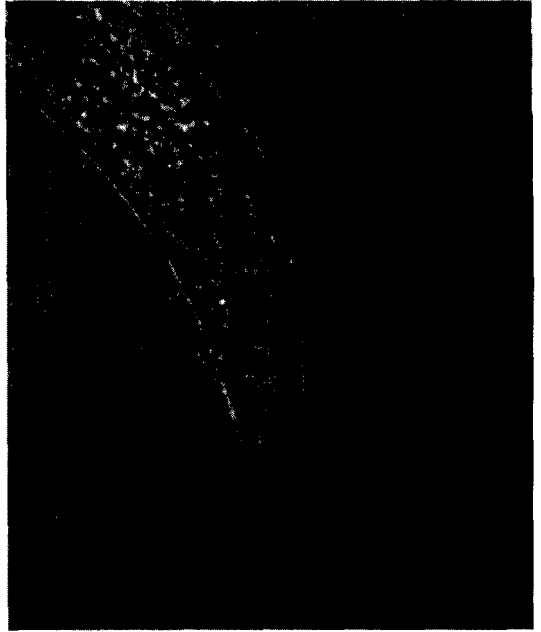
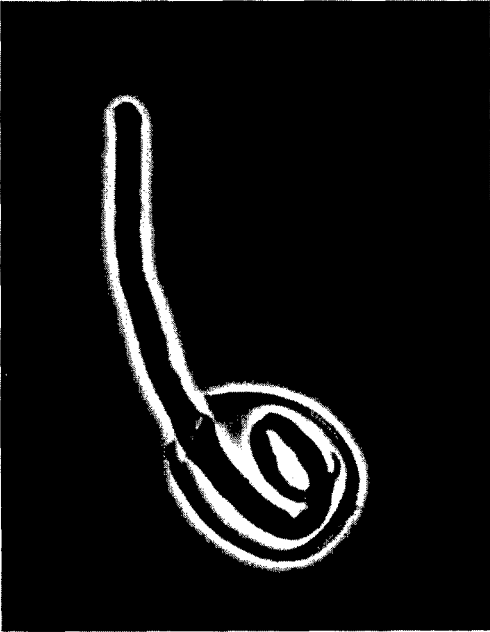
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FIG. 11. First-stage juvenile emerging from egg. Phase-contrast microscopy.

FIG. 12. Encysted third-stage juvenile from experimentally infected grasshopper nymph. Phase-contrast microscopy.

FIG. 13. Second-stage juvenile recovered 10 days after feeding eggs to a grasshopper nymph. Note tail configuration.

FIG. 14. Section of proventriculus showing third-stage juveniles *in situ* within proventricular gland, 7 days post-feeding. Note arrow indicating juvenile within interlobular tissue of glands.



pharyngointestinal junction, visible as a transverse line separating the pharynx from the intestine, was located at the widest point of the body.

Granules were visible at the anterior end of the intestinal lumen and appeared to be more numerous than near the posterior end of the intestine. Under dark-field microscopy, the granules aided in outlining the extremities of the gut. In some specimens, a cuticularized rectum was noticed. In ventral aspect, the anus appeared as a distinct, curved U-shaped slit. The open end of this slit was directed posteriorly.

No evidence of a developed reproductive system was visible under either bright-field or phase-contrast microscopy as noted by Schell (11) in *M. corax*. Seurat (14), however, mentioned a genital primordium near the posterior end of the intestine of presumed third-stage *Tropidocerca* (*Microtetrimeres*) *spiralis*.

In numerous specimens of third-stage juveniles of *M. centuri* mounted in glycerine jelly, a structure somewhat similar to that illustrated by Seurat (14) appeared near the rectal region of the digestive tract (Fig. 10). This may have been the genital primordium. Such a structure was not seen either under phase-contrast or bright-field microscopy in specimens mounted in CMC-10.

No caudal spines or papillae were present. The caudal ends of all individuals at this stage were attenuated (Fig. 10), and terminated in a tiny, unadorned knob ["petit bouton" of Seurat (14)]. This termination is in sharp contrast to juveniles of other tetramerid nematodes, *Tetrameres americana* (3, 4) and *Tropidocerca* (= *Tetrameres*) *fissispina* (1, 14, 15). Posterior ends in these species were blunt and possessed numerous spines which extended beyond the tip.

As shown in a cross-section of a third-

stage juvenile *in situ*, a well-defined cyst isolated the parasite from its host (Figs. 17, 18). This cross-section of the grasshopper showed cysts containing more than one juvenile. Third-stage juveniles recovered in this study were colorless. However, based on non-experimental work Seurat (14) reported that third-stage juveniles of *M. spiralis* were "couleur legerment sanguinolente."

Avian digestive enzymes presumably permitted the release of third-stage juveniles from their cysts. Peristaltic action of the bird's digestive tract may also have aided in this release. After the release of the third-stage juvenile from the cyst it eventually migrated to the bird's proventriculus (Fig. 14). During migration, juvenile nematodes were capable of active movement under the koilin lining of the gizzard. Activity was observed in the gizzard of a laboratory-reared three-day old house sparrow (*Passer domesticus*) infected experimentally 24 hr previously (Figs. 15, 16). Four juveniles were seen under the koilin lining and all moved actively from side to side.

The inability of some infective juveniles to become established in older experimental hosts may have been due to the resistance offered by the koilin lining of the gizzard. For instance, no adult nematodes were recovered from 6 week old chicks and only a few adults were recovered from adult pigeons each of which had been fed about 20 infective third-stage juveniles.

Eight days after the definitive hosts had ingested third-stage juveniles, the width of the juveniles had increased. Other dimensions of the juveniles had not changed greatly.

Dimensions of fixed third-stage juveniles (32-60 days old) recovered from three grasshopper hosts fed eggs from three different avian hosts were (N = number of measurements made):

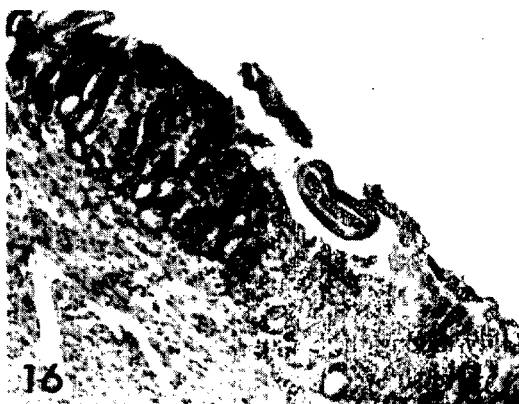


FIG. 15. Sections of *M. centuri* third-stage juveniles in gizzard wall of experimentally infected 72 hour old house sparrow (*Passer domesticus*), 24 hours post-feeding. Anterior end of juvenile penetrating host's mucosa.

FIG. 16. Juvenile beneath koilin lining of gizzard of same section in Fig. 15.

N = 33; length, 1481 μ (1070–1790)

N = 24; width, 49 μ (31–64)

N = 37; buccal capsule length, 13.4 μ
(12.8–15.5)

N = 38; buccal capsule width, 5.4 μ
(5.12–7.7)

N = 27; anus-tail distance, 183 μ (141–206)

Dimensions of fixed, third-stage juveniles (30–34 days old) recovered from three grasshopper hosts fed eggs from only one

avian host were (N = number of measurements made):

N = 84; length, 1539 μ (1122–2040)

N = 86; width, 58 μ (46–100)

N = 82; buccal capsule length, 16.5 μ
(10.4–18.2)

N = 85; pharyngointestinal junction to mouth distance, 488 μ (357–622)

N = 72; anus-tail distance, 158 μ (133–185)

Cysts containing only one juvenile averaged about 500 μ in diameter. Cysts with more juveniles were larger.

FOURTH-STAGE JUVENILES: The minimum length of time for development of fourth-stage juveniles in the experimental or natural definitive host is not known. After feeding encysted juveniles to a one-day old chick (*Gallus domesticus*), an immature female *M. centuri* was recovered in 20 days. Similar attempts to recover fourth-stage juveniles were unsuccessful even though the chicks were sacrificed at periods of time varying from 17 hr to 8 days after feeding them third-stage juveniles. All juveniles recovered from the birds were sexually undifferentiated and, therefore, considered to be still in the third-stage. Seurat (14) gave details of male fourth-stage juveniles of *Tropidocerca* (= *Microtetrameres*) *spiralis*, indicating that sexual differentiation was noticeable during this stage. His work, however, was non-experimental.

DISCUSSION

Only two other experimental reports of juvenile *Microtetrameres* of the various species found in the western hemisphere are known.

Thirty-two to 38 days after feeding eggs of *M. corax* to *Blatella germanica* and larval *Tenebrio* sp., Schell (11) recovered what he believed to be third-stage juveniles from *B. germanica* only. He described them as



FIG. 17. Cross section of grasshopper nymph showing cysts containing third-stage juveniles of *M. centuri* in perivisceral sinus of hemocoel.

FIG. 18. Enlargement of one cyst shown in Fig. 17.

loosely encysted in the insect's hemocoel. He fed these juveniles to one-day old chicks, but 50 to 65 days later the chicks were negative for *M. corax* adults. Cysts containing these juveniles measured 540 to 650 μ in diameter. Encysted specimens measured 2.2 to 2.5 mm long by .060 to .075 mm wide. Their tails were "ball-pointed." Encysted juveniles of *M. centuri* were smaller.

Cram (5) fed eggs of *M. helix* to earth-

worms, pillbugs, two adult grasshoppers (*Melanoplus femur-rubrum* and *M. bivittatus*), several nymphs of *Melanoplus* spp. and to one cockroach (*Blatella germanica*). An unknown number of third-stage juveniles was recovered 26–68 days later from the grasshoppers. However, she recovered only one juvenile from the cockroach and none from the earthworms or pillbugs. Recovered juveniles were larger than third-stage juveniles of *M. centuri* and measured 2.28 to 2.59 mm long by .080 mm wide at their maximum width near the middle of the body. The head was simple with 2 small lips. The mouth cavity measured about 20 μ long. The muscular pharynx measured 172 to 265 μ long and the glandular pharynx measured 530 to 593 μ long. The distance between the anus and the tailtip was 240 to 280 μ . The tip of each tail bore a minute "unadorned ball."

Czaplinski (6), in his survey of nematodes and acanthocephala parasitic in Polish anseriform birds, discussed anatomical variability among these parasites. He found that egg size showed the smallest range of variability of all features studied. Although he did not work with *Microtetrameres*, he did find in some nematodes that egg width is more variable than egg length. He was unable to show any relationship between egg size and age or size of the female nematode producing them or between the egg size and the number of eggs within the female.

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