Growth and Development of Romanomermis culicivorax In Vitro[1]

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Abstract: Various combinations of vertebrate and invertebrate tissue culture and microbiological media were utilized in an attempt to culture *Romanomermis culicivorax* (Mermithidae: Nematoda) in vitro. Most media were unsuitable and caused nematodes to become lumpy, vacuolated, and granular. Slow and limited growth and development of internal structures of the nematodes were obtained with variously supplemented Grace's tissue culture and Schneider's *Drosophila* media. In an enriched Grace's medium, development attained by the nematodes after 3–4 wk was comparable to 4–5-day-old parasites grown in vivo in the mosquito host, *Culex pipiens.* Two molts were observed in vitro. Maximum dimensions in vitro were 7.0-mm length and 87-μm width at the widest point. The stichosome, stichocytes, and trophosome developed prominently. A filiform tail and highly cuticularized tube persisted throughout the culture period in vitro. *Key words: Culex pipiens,* Mermithidae, mosquito nematode.

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Romanomermis culicivorax (Mermithidae: Nematoda) has considerable potential as a biological control agent of several mosquito species (14). Successful in vivo laboratory mass production of this nematode (15) has provided a sufficient quantity of preparasites for large-scale field experiments (3,11,16,17). However, there are practical and economic problems involved in in vivo culture which might be alleviated by in vitro culture (5).

Attempts to grow *R. culicovorax* in vitro have met with limited success. Roberts and Van Leuken (21) obtained growth up to 7.0 mm within 6 wk in Schneider's *Drosophila* medium supplemented with 20% heat-treated fetal bovine serum and 1.5% bovine albumin, in association with Singh's *Aedes aegypti* tissue culture cells; the nematodes survived for 8 wk in the culture medium. Sanders et al. (22) obtained maximum growth of 7.0 mm after 25 days in Schneider's medium with 10% fetal bovine serum; however, molting and gonadial development were not observed. Finney (4) reported 12–14 mm growth and a survival rate of 30% after 6 wk in Grace's medium supplemented with 10% heat inactivated fetal calf serum. No molt occurred, but basic structures developed and all surviving nematodes differentiated into females. The trophosome was observed to be deficient in storage materials.

We report here an attempt to culture the parasitic stages of *R. culicivorax* in artificial media and to compare its in vitro and in vivo growth and development.

MATERIALS AND METHODS

Sand containing the adults and eggs of *R. culicivorax* was provided by Drs. J. J. Petersen and H. C. Chapman of the USDA-ARS Gulf Coast Mosquito Research Laboratory, Lake Charles, Louisiana, and stored at room temperature in plastic bags. Preparasites were collected either by flooding the sand or by extraction using a modified Baermann funnel technique (6). The Baermann technique allowed continuous collection of preparasites for up to a week.

Adults and preparasites collected from sand were washed twice with sterile deionized water and surface sterilized for 1–2 h with an antibiotic-antimycotic solution of 200 U penicillin/ml, 200 μg streptomycin/ml, 25 μg chlortetracyline/ml, 200 U kanamycin/ml, and 1.25 μg fungizone/ml per ml solution (Hansen and Finney, personal communication). This was followed by four sterile distilled water rinses. Surface sterilized adults were placed in 12 × 75 disposable culture tubes containing 1 ml deionized water, 100 U penicillin/ml, and 100 μg streptomycin/ml. After the adults mated and egg laying began, the

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gravid females were transferred daily to new tubes until egg laying stopped. Eggs in the tubes were allowed to develop for 5–7 days. The embryonated eggs were surface sterilized with 0.13% sodium hypochlorite for 5 min followed by five centrifugal rinses. The last rinse contained 100 U penicillin/ml and 100 μg streptomycin/ml per ml solution. Surface sterilized eggs were incubated at 26 C in sterile distilled water or culture medium. The preparasites which emerged from these eggs were utilized for culture work.

The preparasites were transferred to tissue culture petri dishes or flasks containing various combinations of nine commercially available culture media supplemented with different complex chemically defined and undefined substances. The pH and osmotic pressure of the media were adjusted to previously determined optimums of pH 6.4 ± 1 and 240 milliosmoles. The cultures were incubated at 26 C. 10 U penicillin/ml and 10 μg streptomycin/ml per ml medium were routinely added initially to the cultures. Unless otherwise specified, the culture medium was changed or replenished every 2–3 days by withdrawing the supernatant and replacing it with a portion of fresh medium. The following cultural conditions were tested: 1) Incubation of the nematodes directly in liquid medium. 2) Incubation in direct association with growing Singh's *Aedes aegypti* and Hsu's *Culex quinquefasciatus* cell lines. 3) Indirect association with the mosquito tissue culture cells using a special chamber which separated the cells from the nematodes by a 0.22-μm pore filter membrane (23). 4) Incubation in a diphasic medium using 0.50% or 0.85% water agar layer overlaid with the liquid medium. 5) Sephadex neutral beads or glass wool as physical substrates in liquid medium. 6) Incubation at 10% carbon dioxide atmosphere in a candle jar or a carbon dioxide incubator. 7) Aeration of the medium by shaking or by introducing air sterilized by passing through a 0.22-μm pore filter. 8) Utilization of a continuous flow system controlled by a perfusion pump. This system consisted of three 500-ml Erlenmeyer flasks and a 0.45-μm pore filter unit, which were connected in series with a 20-ml screw-cap bottle containing the nematodes. Medical grade silicon rubber tubing was used throughout. All inlet and outlet tubes were pumped at the rate of 0.0195 ml/min with peristaltic pumps. The first flask served as a reservoir from which fresh medium was pumped into a second flask containing growing mosquito cells for conditioning the medium. The conditioned medium was passed through a 0.45-μm pore filter unit, to prevent passage of the insect cells, and introduced into the vial containing 25 preparasites. The depth of the medium in the nematode chamber was maintained at 0.5 cm. The used medium was collected in the third flask. Fresh medium was added to the reservoir flask as required, or the used medium was recycled by passing from the nematode vial to the reservoir flask. In some experiments the tissue culture cells were not used, eliminating the second flask and the filter unit. 9) Intrahemocoelic injection of the preparasites into larvae of *Galleria mellonella* (greater wax moth), *Estigmene acrea* (salt marsh caterpillar), and *Toxorynchitis brevipalpis*.

Second-stage larvae of *C. pipiens* were exposed to preparasites at a host:parasite ratio of 1:12–15 per ml deionized water for 24 h. The mosquito larvae were then washed to remove preparasites which had not infected the larvae. The hosts were kept in an enamel pan at 26 C, aerated, fed daily with mosquito food (60% whole wheat, 25% yeast, 10% blood meal, and 5% nonfat milk), and periodically examined microscopically to follow parasite development. Infected mosquito larvae used for dissection were surface sterilized with 0.52% sodium hypochlorite solution for 15 min, rinsed three times with sterile distilled water, and held for 10–20 min in a previously described antibiotic-antimycotic solution. The mosquito larvae were dissected without rupturing the bacteria-laden gut and gastric caecae, and the released nematodes were placed in culture dishes containing 100 U penicillin/ml and 100 μg streptomycin/ml per ml medium. The nematodes dissected out of the mosquito larvae were incubated in enriched Grace's and Schneider's media to determine whether growth of the parasite would continue outside the mosquito host. Living and fixed nematode

specimens were mounted in a modified Lum's (12) and Ringer's solution containing 1% glucose (7) for microscopic studies. Nematodes were fixed in 3% cold glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.31.

RESULTS

Slow and limited growth and development of internal structures of *R. culicivorax* were obtained when preparasites were incubated in Grace's and Schneider's media supplemented with heat inactivated bovine serum and bovine albumin (GrSA and SchSA, respectively). The maximum growth attained and survival time for selected media combinations are shown in Table 1. SchSA medium stimulated growth of the nematodes when in indirect association with Singh's *A. aegypti* tissue cells, while in GrSA medium growth occurred without the presence of the mosquito cells. Hsu's *C. quinquefasciatus* cell line grew poorly in both of these media. Direct incubation of the nematodes with the mosquito cells was not satisfactory because the host cells outgrew the nematodes and often adhered to them, and interfered with observation. Supplementation of GrSA and SchSA media with varying concentrations of the following did not stimulate growth beyond 3–4 mm (survival time ranged from 1 to 5 wk): chicken plasma, chick embryo extract, horse serum, whole egg ultrafiltrate, fresh liver extract, bovine amniotic fluid, turkey serum, ecdysterone, cholesterol, juvenile hormones, alpha tocopherol, and fatty acids (oleic, linoleic, palmitic, and myristic). Most of these media combinations caused nematodes to become lumpy, vacuolated, and highly granular. Mitsuhashi and Maramorosch's leafhopper medium and SchSA conditioned by growth of *C. quinquefasciatus* and *A. aegypti,* respectively, did not improve growth and survival time of the nematodes, even when used in combination with GrSA. RPMI 1640, NCTC 135, Yunker's medium, McCoy's 5a medium, CMRL 1066, and

Table 1. Maximum length and survival time attained by *Romanomermis culicivorax* in selected culture media combinations.*

Medium†	Maximum length (mm)	Survival time (wk)
Gr + 20% FBS	4.0	4–5
GR + 20% FBS + 1.5% BA (GrSA)	5.0–7.0	7
Gr(50%) + 20% FBS + 1.5% BA	5.0–6.0	7–8
Gr(20%) + 20% FBS + 1.5% BA	3.0–4.0	7–8
Sch + 20% FBS + 1.5% BA (SchSA)	1.5–2.0	1–2
Sch(50%) + 20% FBS + 1.5% BA + Ae	5.5–7.0	7–8
GrSA + Ae	1.5–2.0	1
SchSA + Ae	4.0–5.0	5–7
GrSA + Cq	1.5–2.0	1
SchSA + Cq	1.0–1.5	1–2
Gr(50%) + Sch(50%) (1:1) + 20% FBS + 1.5% BA + Ae	5.5–6.5	7–8
GrSA + BTB, 100 μg, 200 μg/ml	3.5–4.0	6–8
GrSA + Ld, 4 mg/ml	3.5–4.0	5–6
GrSA + Cb, 0.3 μg/ml	3.5–4.0	6–8
GrSA + Tc, 0.5 μg/ml	2.5–3.0	4–6
$GrSA^{++}$	5.5–7.0	6–8
$GrSA^{++}$ + Sch(50%) (2:1) + 20% FBS + 1.5% BA	6.5–7.0	5–7

*Observations based on more than five replicates.

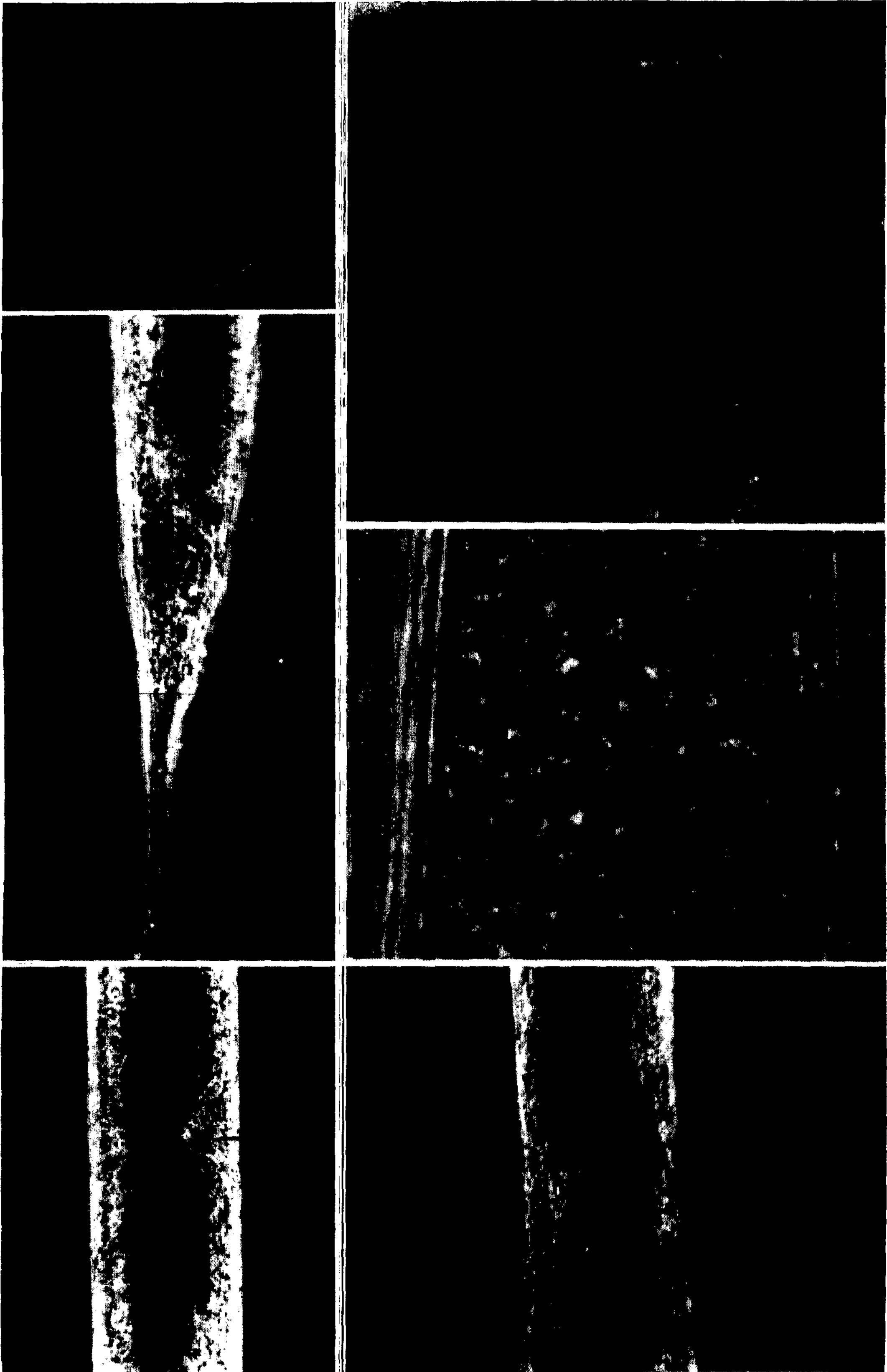
†Abbreviations: Ae, Singh's *Aedes aegypti* tissue culture cell line; Ba, 7.5% bovine albumin, fraction V (Miles); BTB, bacto-tryptose broth (Difco); Cb, Vitamin B12, crystalline (cyanocobalamine) (GIBCO); Cq, Hsu's *Culex quinquefasciatus* tissue cell line; FBS, fetal bovine serum, heat treated (Microbiological Assoc.); Gr, Grace's insect tissue culture medium (GIBCO); GrSA, Gr + 20% FBS + 1.5% BA; $GrSA^{++}$, GRSA + BTB (1.5 mg/ml) + Ld (2 mg/ml) + yeastolate (2 mg/ml) + Cb (0.3 μg/ml) + trehalose (2 mg/ml); Ld, liver digest (PanMede, Paines & Bryne Ltd.); Sch, Schneider's Drosophila medium; SchSA, Sch + 20% FBS + 1.5% BA; Tc, DL alpha tocopherol (ICN Parm.).

Caenorabditis briggsae maintenance medium were not suitable media for *R. culicivorax*. Supernatant from homogenates of postparasitic *R. culicivorax* and *C. pipiens* larvae did not stimulate growth in GrSA medium.

The best growth was obtained with an enriched GrSA medium (Table 1 GrSA^{++}). Newly hatched preparasites averaged 12.5 μm in width and 1.0 mm in length. No changes were observed in the size or morphology of the nematodes until after 2–3 days incubation in the medium when they began to appear shortened and thickened. The nematodes became sluggish and molted for the first time on day 3; molting nematodes appeared to have difficulty in shedding the cast cuticle. On day 4, nematodes measured 1.5–2.0 mm in length; and 2.5–3.0 mm on day 7. A second molt was observed after 14 days incubation when nematodes were 3.5–4.0 mm long. By day 21, they measured 4.0–5.5 mm. The maximum length (7.0 mm) and width at the widest point (87 μm) were attained at day 28; this was a 7-fold increase over the preparasites. In vitro and in vivo growth were essentially the same for the first 3 days. Thereafter, however, in vivo growth was much more rapid, so that at the time of emergence from the host (day 6 or 7), juvenile males measured 7–14 mm and juvenile females 10–18 mm in length.

The development of internal nematode structures began within 3 days of incubation in the culture medium. The posterior end of the nematodes began to shorten at day 3 (Fig. 1) and continued to develop until a filiform tail about 200 μm long had formed during the 2nd wk (Fig. 2). The filiform tail persisted throughout the culture period. Parasites grown in vivo developed a caudal appendage after day 3 which persisted until emergence from the host (Fig. 14). The stylet was lost after the first molt, which occurred on day 3 in the medium. The highly cuticularized tube which extended posteriorly from the stoma was present throughout the lifetime of the nematodes in culture medium (Figs. 4, 9). The structures observed at the anterior end of 2-wk-old cultured nematodes (Figs. 4, 9) were similar to that of 4–6-day-old parasites in vivo (Figs. 11, 13). The stichocytes in the stichosome began to appear at day 2 in both in vitro and in vivo cultures. The stichosome was surrounded by a membrane and consisted of eight pairs of stichocytes initially arranged in rows along the side of the cuticularized tube at the anterior section of the nematode (Fig. 7). The stichosome began to develop at day 2 to day 3, increased in size after day 4, and then began spreading toward the middle section and posterior portion of the body (Fig. 5). Anterior to the stichosome was a distinct granular body with large nucleated cells (Fig. 4). The trophosome originated as scattered globular bodies which began to accumulate in the intercellular spaces between days 3 and 4. These slowly increased in number and spread throughout the middle section up to 0.2 mm from the tail end (Fig. 2). They continued to develop until the 4th wk. The trophosome consisted of these globular storage bodies formed into compact masses with a prominent membrane surrounding the structure (Figs. 3, 6). In in vivo culture the trophosome became prominent at day 3 to 4 after infection (Figs. 10, 12, 14). This obscured the other structures, such as the stichocytes and the gonads. The initiation of the genital primordium occurred at day 3 after infection in vivo (Fig. 10) and continued to differentiate until the emergence of the parasites from the host; the juvenile females exhibited a prominent vulval opening (Fig. 12). In in vitro culture, two juvenile females developed a vulval region (Figs. 3, 8) after the 2nd and 3rd wk of incubation; however sexual maturation never occurred in vitro. Usually after the 4th wk in in vitro culture, the nematodes showed signs of aging; pigments developed along the middle portion of the intestinal region. The body wall did not thicken and was unusually fragile. This was demonstrated when 4–6-wk-old nematodes readily burst during the process of transfer. The stage of the nematodes after growing 3–4 wk in vitro was comparable to 4–5-day-old parasites grown in vivo. Most of the nematodes survived 6–8 wk in culture medium.

Nematodes did not grow beyond 2–3 mm in length when incubated in GrSA^{++} medium under the following cultural conditions: over a water agar layer; use of neutral beads and glass wool; exposure to 10%



carbon dioxide; gassing and shaking of the medium; and utilization of a continuous flow system device. Preparasites injected into nonhost insects (*Galleria, Estigmene,* and *Toxorynchitis*) did not grow or survive in the insect haemocoel.

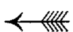
No gonadial development occurred in 1–3-day-old parasites which were dissected from infected mosquito hosts and incubated in culture medium. Older parasites (4–7-days old) developed a genital primordium but no sexual maturation was observed (Table 2).

DISCUSSION

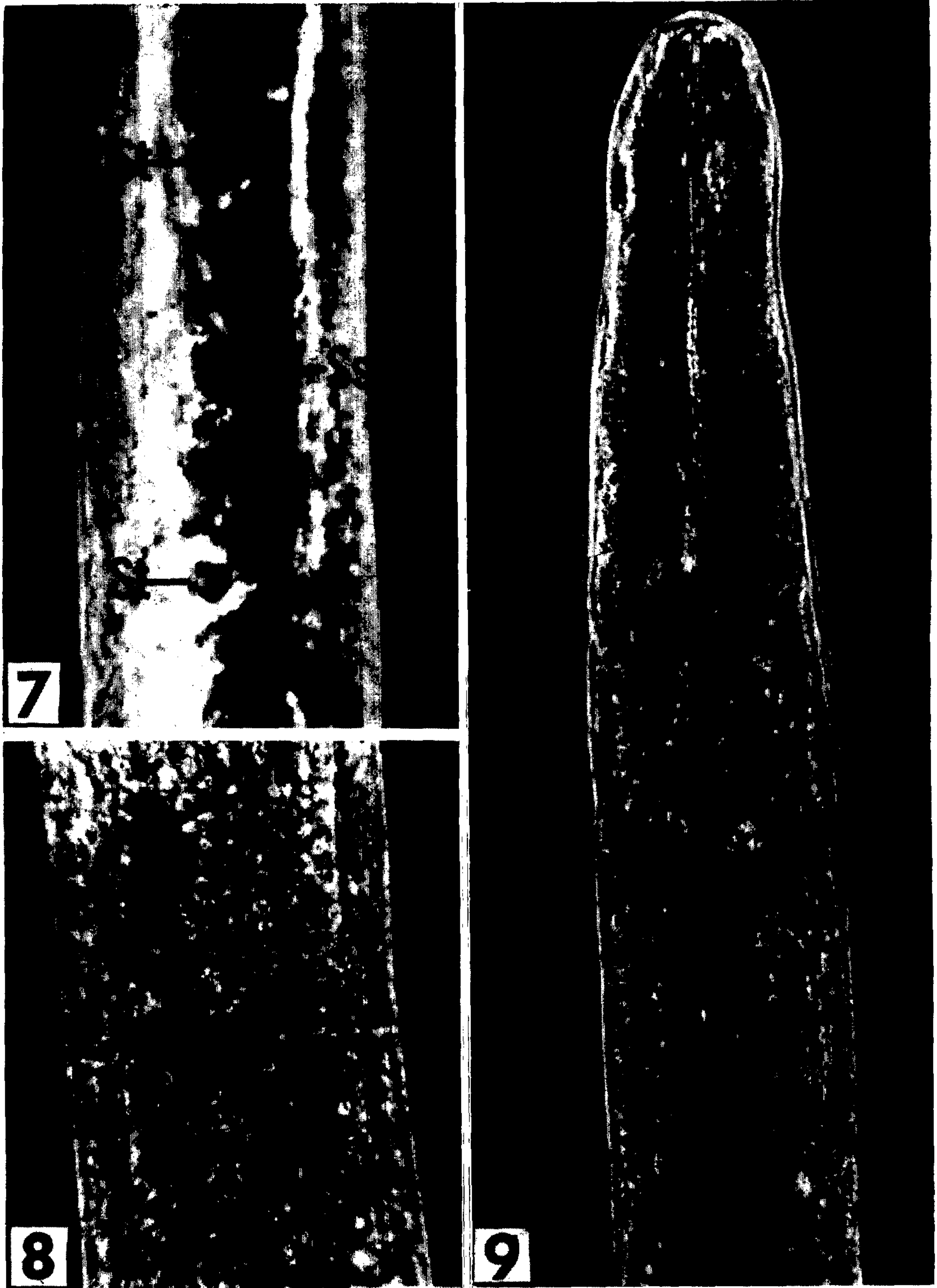
The growth and development of the various structures of *R. culicivorax* cultured in vitro and in vivo were essentially the same during the first 3 days of incubation. Thereafter, the rate of growth and development time was much slower in the artificial media. In contrast, the growth of the parasitic stage in the mosquito larva was rapid after the 3rd day of parasitism up until the time of emergence from the host. Two molts were observed in vitro indicating that a factor was present in the GrSA^{++} medium which promoted some growth but did not stimulate either rapid growth or continuous development of the nematodes. There are four molts in the life cycle of *R. culicivorax* (20). The first occurs in the egg, the second during the parasitic stage inside the host, and the third and fourth molts occur simultaneously in the postparasitic stage. Ultrastructural study of the trophosome in *Gastromermis boophthorae,* a mermithid parasite of blackflies, showed this organ to have structural features adapted for assimilation of nutrients, food storage, and nutrient translocation (2). The trophosome of *R. culicivorax* serves for storage of triacylglycerol, phospholipids, free sterols, sterol esters, glycogen, and proteins (8,9,10). The development in vitro of the trophosome in *R. culicivorax* indicates that the nematode was structurally able to absorb and store to a certain extent nutrients from the culture media. The stichocytes and the stichosome developed prominently in vitro. This may be significant because of the possible role of stichosomes in protein synthesis (18) and the postulated secretion of digestive enzymes by stichocytes (13). Although initial development of a vulval region was observed in a few cases, sexual maturation did not occur in vitro.

Several factors may account for the limited growth and development of *R. culicivorax* under artificial conditions. One factor may be the inability of the nematodes to absorb sufficient nutrients for their biosynthetic and metabolic functions. Also, growth factors required to trigger and maintain continuous development of the nematodes in vitro may not be available in the proper form and concentration. Fetal bovine serum and bovine albumin in Grace's medium provided growth promoting substances which initially stimulated growth; best results in nematode appearance and internal structures were observed when tryptose, liver digest, yeastolate, Vitamin B12, tocopherol, and trehalose were added to this medium. There is no information available on how these complex substances affect the developing nematodes in the culture medium. This is one aspect of study that should be investigated.

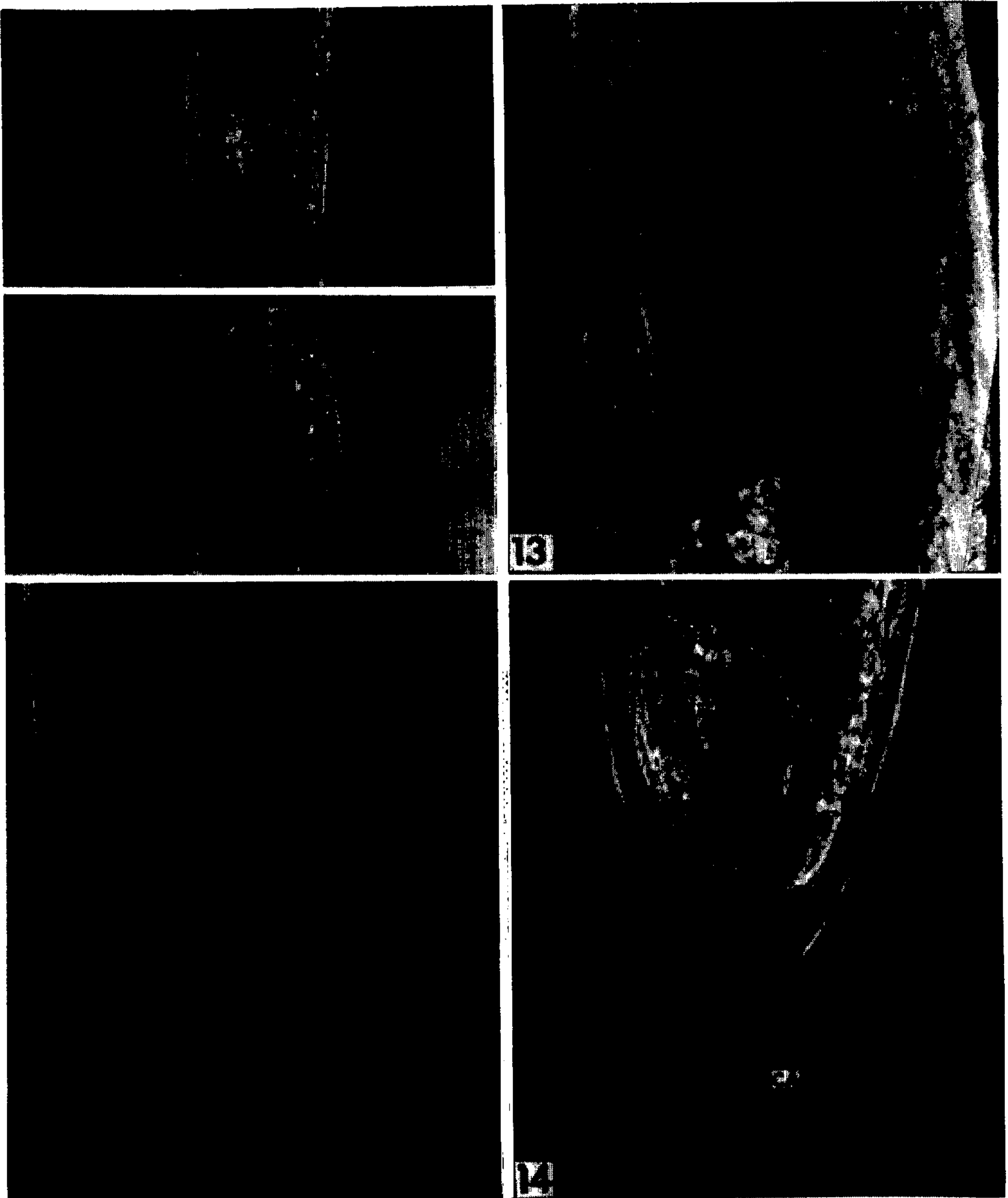
R. culicivorax feeds only during the parasitic stage and absorbs from the host hemolymph all the nutrients that it requires for growth, maturation, and reproduction. In in vitro culture, the nematodes are completely dependent upon the presence and availability of nutrients in the medium. The mechanism by which these nutrients are absorbed by the nematodes from the medium is not understood. Poinar and Hess (19) showed the presence of pores, 70–110 Å



Figs. 1–6. *Romanomermis culicivorax* cultured in vitro (whole mounts). 1) Posterior end of a 2-day-old nematode showing development of a filiform tail (× 560). 2) Posterior end of a 3-wk-old nematode showing filiform tail and the posterior boundary of the trophosome (Ts) (× 512). 3) Developing gonadial region (Gr) of a 4-wk-old nematode. The compact mass of globular bodies, the trophosome (Ts), tends to obscure this area (× 512). 4) Anterior end of a 2-wk-old nematode showing stoma (M), cuticularized tube (Ct), pharyngeal sheath (Ph), and granular nucleated cell (Gs) (× 2000). 5) Middle region of a 10-day-old nematode showing stichocytes (St) (× 1400). 6) A 4-wk-old nematode with developed membrane-bound trophosome (Ts) (× 512).



Figs. 7–9. *Romanomermis culicivorax* cultured in vitro (whole mounts). 7) Anterior section of a 2-day-old nematode showing stichocytes (St) and stichosome (Ss) (× 2000). 8) Middle section of a 16-day-old nematode with developing trophosome (Ts) around the genital primordium (Gr) (× 1056). 9) Anterior region of a 16-day-old nematode showing stoma (M), cuticularized tube (Ct), and stichocytes (St) (× 1800).



Figs. 10–14. *Romanomermis culicivorax* cultured in vivo in *Culex pipiens* larvae (whole mounts). 10) Developing genital primordium (Gr) of a 5-day-old nematode surrounded by trophosome (Ts) (× 1400). 11) Anterior end of a 6-day-old nematode showing stoma (M), pharyngeal sheath (Ph), and cuticularized tube (Ct) (× 700). 12) Vulval region of a 7-day-old nematode showing vaginal opening (V), developing gonads (GP), and compact mass of trophosome (Ts) (× 1120). 13) Anterior section of a 7-day-old nematode showing stichocytes (St), stichosome (Ss), and cuticularized tube (Ct) (× 2920). 14) Posterior end of a 7-day-old nematode with caudal appendage (CA) showing the posterior boundary of the trophosome (Ts) (× 1000).

Table 2. Growth and gonadial development of *Romanomermis culicivorax* dissected from infected *Culex pipiens* larvae after different days in host and incubated in the culture medium.*

Day in host	Medium†	Length at time of dissection (mm)	Maximum length (mm)	Survival time (wk)	Gonadial development‡
1	A	1.0	5.0	2–3	–
	B	1.0	5.0	3–4	–
2	A	1.0	2.5	2–3	–
	B	1.0	4.5	4	–
3	A	1.2–2.0	5.0	3–4	–
	B	1.2–2.0	5.5	2–3	–
4	A	3.0–4.0	6.0–7.0	4	+
	B	5.0	7.5	2–3	+
5	A	4.0–6.0	10.0	2–3	+
	B	5.0	8.0	2–3	+
7	A	8.0–14.0	15.0–18.0	3	+
	B	7.0–14.0	15.0–18.0	3	+

*Based on two replicates of 5–10 male and female parasites each.
†A, Sch(50%) + 20% FBS + 1.5% BA; B, GrSA++; see Table 1 for constituents.
‡–, no gonidial development; +, gonads developed to juvenile stage.

in diameter, in the three membranes which comprise the body surface of *R. culicivorax*. These pores are large enough to allow the passage of nutrients, indicating a possible transcuticular transport system. However, they also observed that the pores in the body wall of parasites incubated in a medium containing heat-treated serum were clogged with deposits, which may block the absorption of sufficient growth factors; this may explain the limited growth obtained in in vitro conditions. The structural adaptation of the body wall to absorb nutrients from the insect host hemolymph during the parasitic existence of mermithid nematodes was substantiated by ultrastructural study done on *G. boopthorae* (1). The hypodermis of the body wall was shown to be connected to the trophosome by cytoplasmic bridges (2). The body wall of *R. culicivorax* grown in vitro appeared visually to be abnormally developed and unusually fragile. Comparative studies on the ultrastructure of the body wall of the nematodes grown in vitro and in vivo would greatly increase our understanding of the role of the body wall in nutrient absorption and transport in *R. culicivorax*.

Similar results were obtained using surface sterilized preparasites and eggs for initiating the in vitro culture. However, the use of eggs was preferred since large numbers of newly-hatched preparasites could be obtained with less manipulation. Although only actively moving preparasites were used, the effects of the antibiotic and sterilant treatment on the viability of the nematodes are unknown. Variable results were observed in nematode growth and survival time when using different lots of sand cultures and batches of culture media. Nematodes were also sensitive to excessive physical disturbance, and agitation incurred during the process of handling the cultures. These are important factors to be considered when attempting to start an in vitro culture. Information on the complex biochemical and physiological relationship between the parasitic mermithid nematode and its mosquito host would be valuable in assessing the nutritional requirements of *R. culicivorax;* this would facilitate the formulation of a suitable medium for continuous growth and maintenance of this nematode in vitro.

LITERATURE CITED

1. Batson, B. S. 1979. Body wall of juvenile and adult Gastromermis boophthorae (Nematoda: Mermithidae): Ultrastructure and nutritional role. Int. J. Parasitol. 9:495-503.

2. Batson, B. S. 1979. Ultrastructure of the trophosome, a food-storage organ in Gastromermis boophthorae (Nematoda: Mermithidae). Int. J. Parasitol. 9:505-514.

3. Brown, B. J., E. G. Platzer, and D. S. Hughes. 1977. Field trials with the mermithid nematode, Romanomermis culicivorax, in California. Mosq. News 37:603-608.

4. Finney, J. R. 1977. The development of Romanomermis culicivorax in in vitro culture. Nematologica 23:479-480.

5. Finney, J. R. 1981. Mermithid nematodes: in vitro culture attempts. J. of Nematology 13:275-280.

6. Goodey, J. B. 1963. Laboratory methods for work with plant and soil nematodes. Ministry Agr. Fisheries and Food Tech. Bull. No. 2, London.

7. Gordon, R., C. H. Bailey, and L. W. Barber. 1974. Parasitic development of the mermithid nematode Reesimermis nielseni in the larval mosquito Aedes aegypti. Can. J. Zool. 52:1293-1302.

8. Gordon, R., W. J. Condon, and J. M. Squires. 1980. Sterols in the trophosomes of the mermithid nematodes Neomesomermis flumenalis and Romanomermis culicivorax relative to sterols in the host hemolymph. J. Parasitol. 66:585-590.

9. Gordon, R., J. R. Finney, W. J. Condon, and T. N. Rusted. 1979. Lipids in the storage organs of three mermithid nematodes and in the hemolymph of their hosts. Comp. Biochem. Physiol. 64B:369-374.

10. Ittycheriah, P. I., R. Gordon, and W. J. Condon. 1977. Storage material of the nematode Romanomermis culicivorax, a mermithid parasite of larval mosquitoes. Nematologica 23:165-171.

11. Levy, R., and T. W. Miller. 1977. Experimental release of Romanomermis culicivorax (Mermithidae: Nematoda) to control mosquitoes breeding in Southwest Florida. Mosq. News 37:483-486.

12. Lum, P. L. 1961. The reproductive system of some Florida mosquitoes. 1. The male reproductive tract. Ann. Entomol. Soc. Am. 54:397-401.

13. Muller, G. W. 1931. Uber Mermithiden. Zeitschrift fur Morphologie und Okologie der Tierre. 24:82-147.

14. Petersen, J. J., and H. C. Chapman. 1979. Checklist of mosquito species tested against the nematode parasite Romanomermis culicivorax. J. Med. Entomol. 15:468-471.

15. Petersen, J. J., and O. R. Willis. 1972. Procedures for the mass rearing of a mermithid parasite of mosquitoes. Mosq. News 32:226-230.

16. Petersen, J. J., and O. R. Willis. 1972. Results of preliminary field application of Reesimermis nielseni (Mermithidae: Nematoda) to control mosquito larvae. Mosq. News 32:312-316.

17. Petersen, J. J., and O. R. Willis. 1976. Experimental release of mermithid nematode to control flood water mosquitoes in Louisiana. Mosq. News 36:339-342.

18. Poinar, G. O., Jr., and R. Hess. 1974. Anatomy of the preparasitic stage of Hydromermis conopophaga (Mermithidae: Nematoda). J. Nematol. 6:67-74.

19. Poinar, G. O., Jr., and R. Hess. 1977. Romanomermis culicivorax: morphological evidence of transcuticular uptake. Exp. Parasitol. 42:27-33.

20. Poinar, G. O., Jr., and W. A. Otieno. 1974. Evidence of four molts in the Mermithidae. Nematologica 20:370.

21. Roberts, D. W., and W. Van Leuken. 1973. Limited growth of a mermithid nematode cultured in association with tissue culture cells. Abstr. Int. Colloq. Insect Pathol. Microbiol., Oxford, England. p. 91.

22. Sanders, R. D., E. L. R. Stokstad, and C. Malatesta. 1973. Axenic growth of Reesimermis nielseni (Nematoda: Mermithidae) in insect tissue culture media. Nematologica 19:567-568.

23. Shapiro. M., and D. W. Roberts. 1976. Growth of Coelomomyces psorophorae mycelium in vitro. J. Invert. Pathol. 27:399-402.