Development of Thelastoma bulhoesi (Oxyurata: Thelastomatida) and the Effect of Thiabendazole on the Unembryonated Egg

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Abstract: The embryological and postembryological development of *Thelastoma bulhoesi* was determined. Initial cleavage was into unequal cells and occurred within 1–2 hours at 25 C. Cell division was holoblastic but no true morula is formed. Gastrulation occurred at approximately 48 hours by epibolic synectic mechanisms. First-stage larvae were fully developed at 96 hours. The molt to second-stage larvae was initiated in the egg and was completed at hatching. Second-stage larvae were first observed in the host at 11 hours postinfection, third-stage larvae at 18 hours, and fourth-stage larvae at 192 hours. Adult female worms were observed at 32 days. Thiabendazole, in even the lowest concentrations, inhibited the development of unembryonated ova. *Key words:* development, ovicide.

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Dobrovolny and Ackert (6) gave a generalized account of the embryology of *Leidynema appendiculata* with illustrations of the prevermiform stage, embryonated stage, and the infective stage. They also described the unusual, nonmotile infective stage and pointed out that *Leidynema* has holoblastic, but unequal, cleavage. They interpreted the cessation of activity of the first-stage larva as a molting activity. This is probably correct as demonstrated by Todd (11) for *Hammerschmidtiella diesingi* who also observed one molt within the egg, with the cuticle retained, and a second molt just prior to, or at the time of, hatching. Dobrovolny and Ackert (6) do not identify the number of postembryonic stages, but left the impression that there were four larval stages after hatching. The interpretation of molting by Todd was not borne out in the research by Cali and Mai (3) who describe second-, third-, and fourth-stage larvae of *Blatticola blattae* free in the gut of the host.

Larvicidal and ovicidal activity of thiabendazole (TBZ) has been reported by several researchers (2,4,7). McCallister (8) demonstrated that the drug was ovicidal only for unembryonated eggs of *Haemonchus contortus* although exposure never occurred in the single-cell stage. Fully embryonated eggs did not appear to be affected. He suggested that TBZ may interfere with cell division.

The above are the only descriptions of embryonic or postembryonic development for pinworms of cockroaches. The embryology has not been described for any member of the genus *Thelastoma,* nor have any larval stages been described. There also appears to be confusion on the timing of the molts. The embryology and postembryological development of *Thelastoma bulhoesi,* a parasite of *Periplaneta americana,* is described and the effects of thiabendazole on egg development after exposure at the unembryonated stage are reported.

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MATERIALS AND METHODS

In vitro determination of embryological development: Three adult female worms removed from a cockroach hindgut were placed in depression slides with sterile 0.75% NaCl and macerated with a needle to liberate unembryonated ova from the uterus. These glass slides were placed on top of glass shreds (to elevate the slide) in 15 × 90-mm petri plates with moist filter paper in the bottom. With the petri dish cover replaced this became an oxygenated incubation chamber that would impede evaporation. The eggs were cultured at 25 C and examined at 10 × magnification at 1-hour intervals from 0800 to 2400 hours for 6 days. The time required to reach typical stages was recorded, and drawings (using a Leitz camera lucida) were made of live stages in physiological saline.

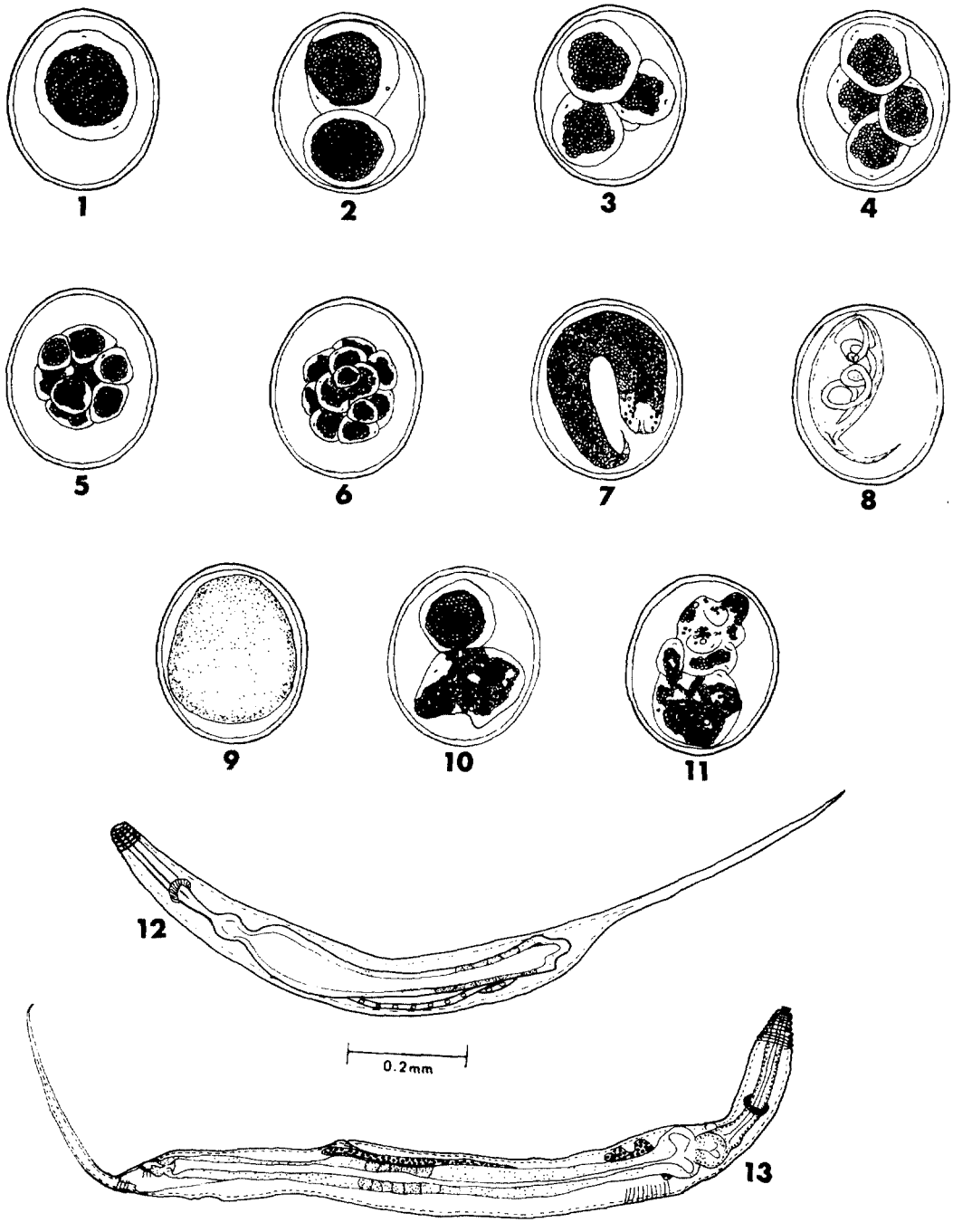
In vitro inhibition of unembryonated eggs by TBZ: Enembryonated eggs were exposed to thiabendazole (wettable powder) at concentrations (wt./vol.) of 0.25%, 0.12%, 0.06%, and 0.03% for 24 hours, 48 hours, and 96 hours. A saturated solution of the drug was prepared by mixing 28 g of TBZ in 100 ml 0.75% NaCl at pH 7.0 and agitating for one hour. The excess powder was then removed by centrifugation. The supernatant fluid was used in each trial for the same times indicated. This soluble TBZ was diluted 1:1 for another test concentration. Since thiabendazole is 3.84% soluble in water at pH 2.2 and decreases in solubility above and below this point (9), the soluble level of thiabendazole used in the current study was well below this amount. Brown (2) has shown that TBZ is soluble in water as follows: 0.13 mg/ml at pH 4.4 and 0.02 mg/ml at 8.2. All trials were conducted at pH 7.0 and at 25 C.

Eggs were exposed to TBZ in wet mounts as described previously for the embryology studies in 0.75% NaCl solution mixed with TBZ at the concentrations noted above. The number of eggs having reached the one-, two-, and three-cell stage, and the morula or gastrula stages were counted at 24, 48, and 96 hours. Drawings by camera lucida were made of typically deformed embryos.

In vivo determination of postembryological development: Artificial infections were established in *P. americana* reared from detergment-washed oothecae by a method similar to that of Cali and Mai (3). Adult cockroaches were chilled for 1 hour at 10 C and fastened to a microscope slide (ventral surface up) with masking tape. Twenty-day-old embryonated eggs were mixed with honey, and a drop of this mixture was placed underneath the labrum of the cockroach with a No. 1 insect pin. The feeding procedure was carried out underneath a dissecting microscope. Cockroaches thus infected were isolated in finger bowl cages, provided food and water, and sacrificed and examined for eggs, larval stages, and adults at 11, 12, 14, 18, 24, 36 hours; 2, 4, 8, 16, and 32 days. Larval stages were heat killed, fixed in formaldehyde, illustrated, and the prepatent period approximated.

RESULTS

In vitro embryological development: Figures 1 through 8 show characteristic stages observed in the normal embryology of *T. bulhoesi*. Single cell ova (Fig. 1) divide transversely into two cells (Fig. 2) of unequal size within 1–2 hours at 26 C. A single polar body is sometimes visible at this stage. Beginning with the second cleavage, each of these two cells have their own cleavage rhythm. This does not appear to be due to size, however, as the larger cell divides first and thereafter independently but equally as fast. This second cleavage results in a transitory triangle, or three-cell stage (Fig. 3). The smaller bastomere will divide into posterior and anterior daughter cells forming a T-shaped figure. The bastomere at the base of the T then migrates to one side to form the classic rhomboid figure after approximately 12 hours (Fig. 4). The above divisions are holoblastic, but not really radial or spiral. Therefore, no true morula stage is formed. Herein, morula is defined as approximately the six- to eight-cell stage and occurs at about 24 hours (Fig. 5). The blastocoele is very small and difficult to detect, but usually can be seen at the 12-to 16-cell stages. Gastrulation (Fig. 6) occurs at approximately 48 hours by epibolic synectic mechanisms; that is, the ectoderm appears to grow over the endo-



Figs. 1–13. Embryonic and postembryonic development of *Thelastoma bulhoesi*. 1). Unembryonated egg, single-cell stage. 2) Two-cell stage. 3)Three-cell stage. 4) Four-cell stage. 5) Morula. 6) Gastrula. 7) First-stage larvae. 8) Infective stage (larvae in first molt). 9–11) TBZ exposed embryos. 12) Third-stage larvae. 13) Fourth-stage larvae.

derm without a change in cell positions. It is seldom possible to count the cells at this stage. First-stage larvae are fully developed by about 64 hours. Motility may begin as early as 60 hours, just after gastrulation. Figure 8 shows the resting stage, within the egg, which has undergone the first molt to the second-stage larva, the first-stage larval sheath being evident. Cleavage in nematodes is determinant: the smaller blastomere at the two-cell stage and its progeny become the head end of the larvae while the larger blastomere will form the tail. Table 1 shows the percentage of eggs in various stages of development after 1, 2, 4, 8, 16, 32, 64, and 128 hours in culture. Five percent of the eggs failed to develop properly. This occurred within the first hour or two. Those remaining all developed completely, so this statistic is not listed in the table.

The effect of thiabendazole on ova development is shown in Table 2. Development was impeded at minimum solubility and time. Figures 9, 10, and 11 demonstrate some of the aberrant forms observed.

Figures 12 and 13 portray female third- and fourth-stage larvae, respectively. Second-stage larva was first observed at 11 hours postinfection; third-stage larvae at 18 hours, and fourth-stage larvae at 192 hours. Adult female worms were observed in infected cockroaches at 32 days. The sequence of events and the time required to complete them are summarized in Table 3. All larvae and adult female worms were recovered from the anterior one-third of the hindgut.

Table 1. Mean percentage of eggs of *Thelastoma bulhoesi* in each stage of development after a given number of hours in 0.75% NaCl, pH 7.0, at 25 C.

	Stage of development*								
Hours	1	2	4	8	16	G	PV	L_1	R
0	100	0	0	0	0	0	0	0	0
1	94	1	0	0	0	0	0	0	0
2	43	49	3	0	0	0	0	0	0
4	1	27	52	15	0	0	0	0	0
8	0	8	24	24	37	2	0	0	0
16	0	0	0	0	15	77.5	2.5	0	0
32	0	0	0	0	11.2	54.8	29.0	0	0
64	0	0	0	0	0	0	4	91	0
128	0	0	0	0	0	0	1.1	1.1	92.8

*1 to 16 = number of cells in embryo, G = gastrula, PV = prevermiform, L_1 = first-stage larvae, R = resistant.

Table 2. Effect of thiabendazole (TBZ) on development of *Thelastoma bulhoesi* eggs. Percentage of eggs at each stage of development after 96 hours in 0.75% NaCl, pH 7.0, and in various concentrations of thiabendazole (TBZ).

Concentration of TBZ	Stage of development					
	Disrupted	1-cell	2-cell	Rhomboid 3-cell	Morula	Gastrula
Control	2	0	0	0	23	75
Sol./2*	3	3	30	47	16	0
Soluble+	61	29	3	7	0	0
0.03‡	53	39	4	4	0	0
0.6‡	91	9	0	0	0	0
0.12‡	100	0	0	0	0	0
0.25‡	100	0	0	0	0	0

*Soluble level of the TBZ at pH 7.0, 26 C diluted 1:2 in 0.75% NaCl.
+Soluble level of the TBZ at pH 7.0, 26 C.
‡Weight/Volume %.

Table 3. Stages of postembryonic development of *Thelastoma bulhoesi* and the time of their appearance postinfection within *Periplaneta americana* (+ = present, − = not observed).

Time (hours)	Stage of development*			
	EE (L_2)	L_3	L_4	Adult
11	+	−	−	−
12	+	−	−	−
14	−	−	−	−
18	−	+	−	−
24	−	+	−	−
36	−	+	−	−
48 (2 days)	−	+	−	−
96 (4 days)	−	+	−	−
192 (8 days)	−	−	+	−
384 (16 days)	−	−	+	−
768 (32 days)	−	−	−	+

*EE = embryonated egg, L_2 = second-stage larvae, L_3 = third-stage larvae, L_4 = fourth-stage larvae.

DISCUSSION

Thelastoma bulhoesi appears to follow a pattern of embryological development similar to other nematodes. Determination of the ultimate fate of cells is difficult, as the adult presumably develops only within the host. It appears that the smaller blastomere resulting from the first mitotic division is probably the parental germinal cell (P_1) and will eventually give rise to the anterior end of the larvae. The larger blastomere is the first somatic cell (S_1) and will give rise to the proctodeum. This hypothesis needs verification. Mitosis is delayed in the P_1 cell until the S_1 has divided to form a three-cell stage. This is similar to *Meloidogyne naasi* according to Siddiqui and Taylor (10). The process of gastrulation observed in *Thelastoma bulhoesi* also appears to be similar to that of *M. naasi,* and to that of *Nacobbus serendipiticus* according to Clark (5). Configurations of the embryo and developmental time agree with the above authors.

Apparently all embryos do not develop uniformly. The first cell division occurs nearly simultaneously, but the second and subsequent divisions are increasingly asychronous until, at about 8 hours, the embryos may be in any one of five stages. However, at about 64 hours nearly all embryos are once again at the same stage. Life tables of nematode eggs development also show this asynchrony. However, the development time and extent of synchrony are functions of the stages one chooses to recognize. Other alternative explanations are 1) the rapid metabolism of the actively dividing cells is causing disruptions, such as temporary O_2 competition, etc., in the microenvironment, or 2) the rate of cell division is genetically determined and has evolved so that eggs develop at variable rates to help insure the survival of some offspring. Since not all embryos are synchronous again at 64 hours this is probably not a survival mechanism.

In the present study the unembryonated egg proved to be susceptible to thiabendazole. This is in agreement with the findings of Egerton (7) on various helminth species. Changes in pH, which might have been induced by mixing TBZ, do not affect embryological development (unpublished data). Most nematode eggs have a lipid layer incorporated into one of the layers of the shell. This makes the shell somewhat impermeable to polar solvents (1). It was obvious (Figs. 9–11) that thiabendazole interfered in some way with cell membrane synthesis and/or cytokinesis. As most nematodes do not undergo further cell division when developing from second-stage larvae to adult (1), this would explain why the drug affects only the unembryonated eggs, as was found by McCallister (8).

Postembryonic development is similar to that of other pinworms and the Ascaridata with which they have been classified in the past. The first-stage larva undergoes a molt

to the second-stage larva within the egg and prior to ingestion by a host. As only two larval stages and the adult were ever observed in the intestine, it is assumed that the molt from L_2 to L_3 occurs shortly after the time of hatching; this would agree with Todd (11). Hatching occurs through a small, indistinct operculum. This operculum becomes visible with treatment of the eggs with one percent trypsin, although artificial hatching has not been achieved. The first adult females were observed in 32 days, and this is proposed as the first determination of the prepatent period of this worm.

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