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Growth and Morphological Changes of *Strongyloides ratti* in Rats

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Since the first description by Sandground (1925), the life cycle and morphology of *Strongyloides ratti* have been studied by various workers. These studies were carried out on the premise that the percutaneously or subcutaneously inoculated third-stage larvae (L_3) migrated to the lung from the skin and finally reached the small intestine in rats (Abadie, 1963; Wertheim and Lengy, 1965) and mice (Dawkins and Grove, 1981). In contrast, Abe (1964) found that this worm migrated into the head then went down to the small intestine. Hattori *et al.* (1968) showed that the larvae rarely migrated to the lung before their intestinal phase. We reported a detailed study on the migration route, which revealed that the larvae passed through the loose subcutis to the cranial cavity and naso-frontal portion of the head, and eventually reached the small intestine (Tada *et al.*, 1979). Recently, Murrell (1980) reconfirmed the presence of above pathway. Hattori (1981) observed the cranial migration occurring even after percutaneous infection at abdomen and tail.

Thus the clarification of the migration route via the head prompted us to re-evaluate the morphological changes of *S. ratti* during the course of migration in rats.

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Materials and Methods

Host animal and infection

Male Wistar rats weighing 200–230 g were used. The strain of *Strongyloides ratti* was that used in previous study (Tada *et al.*, 1979) which has been maintained in our laboratory by successive passage in Wistar rats for more than 6 years. L_3 were harvested from filter paper cultures after 4 day incubation at 27 C and washed several times with physiological saline. Rats were inoculated subcutaneously at the groin with 3,000 L_3 suspended in about 0.5 ml physiological saline.

Recovery of worms

Five rats at each autopsy were starved for 12 hours to avoid food residue in the small intestine and were bled under ether anesthesia. The necropsies were made from 48 hours to 102 hours post inoculation (p.i.). The worms were recovered from the following teased tissues and organs; cranial cavity, nasofrontal portion (nasal cavity and the major portion of the maxilla) and small intestine. The upper half of small intestine was longitudinally opened in order to promote the emergence of parasites. These tissues and organs were incubated in Petri dish with physiological saline at 37 C for about 2 hours. All the worms emerged

from the tissues and organs were counted and collected under a dissecting microscope. Based on the stage of development described by Wertheim and Lengy (1965), the parasites recovered were classified into third-stage larva (L_3), fourth-stage larva (L_4) and adult.

Worm morphology

Worms of different stages were recovered from following locations in the rats: L_3 before inoculation (0 hour), from the subcutis (10 hours p.i.), cranial cavity (24 hours p.i.), naso-frontal portion (48 hours p.i.) and small intestine (48 hours p.i.); L_4 from the small intestine (3 days p.i.); and adults from the small intestine (4, 6, 8, 10, 12, 15, 17 and 20 days p.i.).

Recovered worms were killed in hot water (1 minute at 60 C) and were stained with dilute Lugol solution. The contour and location of anatomical landmarks of 30 worms from each group were drawn with the aid of Camera lucida. The following dimensions of the worm were measured: length of body, esophagus, intestine, tail, genital primordium of L_3, the space occupied by the reproductive system in L_4 and adult, body width at its maximum and at anus, and distance between head tip and nerve ring. The number of eggs in the reproductive system was also counted when visible.

Statistical analysis was performed by Student's t test. A value of $p<0.05$ was considered to be significant.

Results

Recovery of worms

The number of recovered worms from the cranial cavity, naso-frontal portion and small intestine at each autopsy is summarized in Table 1. At 48 hours post inoculation, the worm counts (L_3) in the cranial cavity and naso-frontal portion were much higher than that in the small intestine, while it decreased markedly with the lapse of time. In contrast, the number of worms (L_3, L_4 and adult) in the small intestine showed an increase throughout the whole experimental period and reached the peak, 346.8±121.3, at 102 hours p.i.. The stage composition of worms, L_3: L_4: Adult, in the small intestine is graphically shown in Fig. 1. The whole population recovered by 60 hours was mostly occupied by L_3, and thereafter L_4 appeared gradually. At 66 hours p.i., about a half of the recovered population was L_4, and they were gradually

Table 1 The recovery of *Strongyloides ratti* from the cranial cavity, naso-frontal portion and small intestine of rats inoculated subcutaneously with 3,000 L_3

Time (hrs.) post incoulation	No. of worms recovered ($\bar{X}$±SD)/rat*		
	Cranial cavity	Naso-frontal portion	Small intestine†
48	166.6±81.9	384.8±137.6	91.4± 32.3
54	65.4±34.9	138.0± 78.7	142.0± 61.2
50	44.2±20.1	159.6± 67.3	120.6± 38.9
66	9.2±10.6	70.0± 34.0	268.6±133.5
72	10.0± 5.8	70.0± 20.0	174.4± 52.0
78	7.0± 5.4	19.2± 5.9	202.0± 74.5
84	4.4± 3.4	15.8± 20.0	196.4± 75.8
90	1.4± 1.1	17.6± 7.9	203.8±101.5
96	1.2± 1.3	6.8± 2.6	251.6±102.6
102	1.2± 1.3	2.4± 1.8	346.8±121.3

* 5 rats per necropsy.

† All the stages of *S. ratti* (L_3, L_4 and adults) are included.

Table 2 Comparison of the size ($\bar{x}\pm SD$) of *Strongyloides ratti* third-stage larvae (L_3, 30 specimens each) recovered from various portions in rats arrenged by post inoculation hours

Time (hrs.) post inoculation	Body length (μm)	Body width (μm)	Distance between head tip and nerve ring (μm)	Esophagus length (μm)	Intestine length (μm)	Tail length* (μm)	Body width at anus (μm)	Anal ratio†	Length of genital primordium (μm)
0 (before inoculation)	642.3±31.2	16.3±1.8	99.2± 7.7 (15.4%)‡	281.5±11.7 (43.8%)	294.6±21.6 (45.9%)	66.3±2.9 (10.3%)	10.6±1.1	6.25	11.8±1.9
10 (in the subcutis)	651.3±40.0	19.6±2.0	106.3± 7.3 (16.3%)	285.5±16.0 (43.8%)	292.9±24.3 (45.0%)	71.9±4.7 (11.0%)	13.9±2.0	5.17	15.5±1.9
24 (in the cranial cavity)	664.5±41.6	19.5±1.2	103.0±13.2 (15.5%)	294.4±18.3 (44.3%)	298.1±25.4 (44.9%)	72.0±4.7 (10.8%)	12.5±1.1	5.76	16.2±3.2
48 (in the naso-frontal portion)	687.1±34.2	18.5±0.9	99.8±11.7 (14.5%)	332.1±28.4 (48.3%)	284.0±23.0 (41.3%)	71.0±4.9 (10.3%)	11.6±0.9	6.12	19.7±4.2
48 (in the small intestine)	763.4±29.9	21.4±2.5	106.7± 7.8 (14.0%)	393.4±19.4 (51.5%)	292.9±22.2 (38.4%)	76.2±4.9 (10.0%)	16.2±2.4	4.70	31.6±5.2

* Distance between anus and the tail tip.

† Tail length/body width at anus.

‡ Percentage in the parenthesis shows the relative length per whole body length.

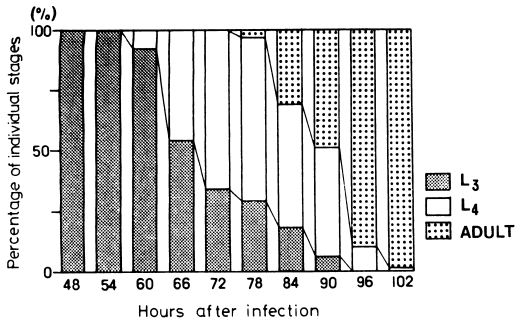

Fig. 1 The population composition (L_3 : L_4: Adult, 100 in total) of *Strongylrides ratti* recovered from the small intestine of rats during the course of infection.

replaced by adults. At 96 hours and later, most of the worms were young and mature adults.

Worm morphology

As summarized in Table 2, the mean body length of L_3 before inoculation was 642.3±31.2 μm, while it slightly increased to 687.1±34.2 μm in L_3 from the naso-frontal portion and 763.4±29.9 μm in L_3 recovered from the small intestine. It was noted that in comparison with the wedge-shaped head of L_3 before inoculation (Fig. 2-a 1), the anterior end of L_3 from the cranial cavity, naso-frontal portion and small intestine was round or "bulb-like" in shape (Fig. 2-a 2). Moreover, some L_3 from the small intestine contained distinct droplets under the cuticle (Figs. 2-a 3). A significant elongation occurred in the relative length of esophagus, from 43.8% in the initial L_3 to 51.5% in the late L_3 from the small intestine at 48 hours p.i. ($p<0.001$). The anal ratio (tail length/body width at anus) was reduced in the parasites from the small intestine in comparison with pre-intestinal stages. The length of genital primordium in L_3 increased with time (Table 2 and Figs. 2-b 1, 2, 3).

The measurements of various dimensions of L_4 and adults are illustrated in Fig. 3 and Table 3. The length of space occupied

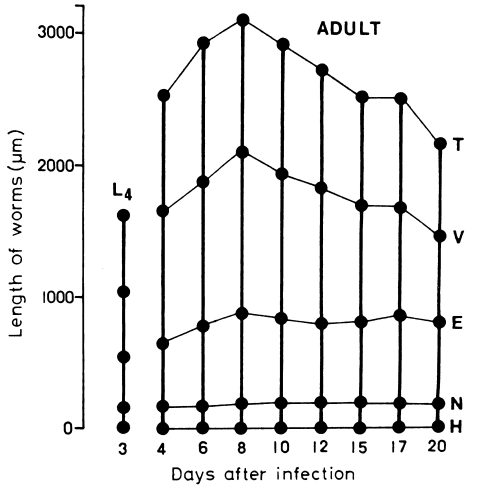

Fig. 3 The changes in sizes and structures of *Strongyloides ratti* fourth-stage larvae (L_4) and adults recovered from the small intestine of rats during the course of infection (Measurements were made on 30 specimens).
H, head tip; N, nevre ring; E, the posterior end of esophagus; V, vulva; T, tail tip.

by reproductive system of L_4 was about one half of that of adult and no egg was found in it (Table 3). The body length of adults increased until 8 days p.i. and thereafter it tended to decrease. On day 20, the body length reduced to about two thirds of the maximum at day 8, while no change was seen in esophagus length (Fig. 3). A reduction of reproductive system was seen after 10 days p.i., as seen in the size of reproductive system, from 2069.5±160.3 μm on day 8 to 931.1±138.2 μm on day 20. The fecundity also reduced with the atrophy of reproductive system (Table 3).

Discussion

This study clarified the growth and morphological changes occurring in *S. ratti* during the course of infection in rats, with special reference to migration through the head tissue.

Larvae moulted two times in the upper small intestine. The timing of the moult-

Table 3 Chronological changes in the various dimensions and the fecundity of *Strongyloides ratti* fourth-stage larvae (L_4) and adults recovered from the small intestine of rats

Days post inoculation	Parasite stage*	Body length ($\bar{X}\pm SD$, μm)	Length of the space occupied by reproductive system ($\bar{X}\pm SD$, μm)	Reproductive system ratio (%)†	No. of eggs in reproductive system ($\bar{X}\pm SD$)
3	L_4	1537.1± 97.1	473.6± 73.4	30.8	0
4	Adult	2554.8±107.8	1642.3± 79.7	64.3	5.9±1.7
6	Adult	2908.3±168.2	1896.5±162.1	65.2	6.8±1.8
8	Adult	3129.5±564.3	2069.5±160.3	66.1	8.2±2.4
10	Adult	2903.7±449.4	1831.6±155.6	63.1	6.2±1.5
12	Adult	2749.4±235.3	1654.0±190.6	60.2	5.1±1.7
15	Adult	2545.3±199.3	1457.6±180.6	57.3	3.7±1.5
17	Adult	2526.2±291.1	1391.1±275.3	55.1	2.6±2.0
20	Adult	1995.6±174.5	931.1±138.2	46.7	0.2±0.6

* 30 specimens each.

† Percentage of the reproductive system to the whole body length.

ing itself in the small intestine was approximately the same to that reported by Abadie (1963) in whose experiment larvae were considered to migrate through the lung. The body length and relative esophagus length were significantly enlarged in L_3 obtained from the head tissue compared with that of L_3 before inoculation. This result apparently coincides with previous report by Wertheim and Lengy (1965) who described about 5% increase in the larvae recovered from the lung. As are reported recently, however, the importance of lung migration of *S. ratti* larvae remains quite equivocal. Nojima *et al.* (1981) found the elongation of esophagus when larvae reached the head tissue, while no increase in the body length occurred.

Bonner (1979) demonstrated that the head of L_3 of *Nippostrongylus brasiliensis* exhibited "bulb-like" shape following development in the lung of rats. Present finding on the change of head shape in *S. ratti* L_3 is analogous to this evidence.

Bird and Rogers (1965) reported that during the moulting of *Meloidogyne javanica,* the dissolution of the inner layer of the old cuticle occurred and that the space between old cuticle and new one became filled with droplets. Similar droplets seen in our specimens, intact and stained, would be also reflecting moulting process to the L_4 stage.

Abe (1964) briefly reported the growth of genital primordium in L_3 recovered from the tissues at 40 hours p.i. On the contrary, other investigators reported no increase in size of genital primordium of L_3 recovered from the lung (Abadie, 1963; Wertheim and Lengy, 1965). In the present study, it was noted that the genital primordium enlarged even in the early migrating stage through subcutis.

The adult *S. ratti* actually undergoes a dramatic reduction in its length and width during the later stage of a primary infection in the small intestine of rats (Moqbel and Denham, 1977; Moqbel and McLaren, 1980). Our measurements of the worms at the terminal stage of infection, 17–20 days p.i., suggest that the reduction of the body length may partially account for the atrophy of reproductive system.

Summary

Because of the recent revelation that *Strongyloides ratti* migrates through the rat's head in its migration to the small intestine, a morphological study was per-

formed on *S. ratti* during the course of infection. The third-stage larvae (L_3) inoculated subcutaneously migrated to the cranial cavity and naso-frontal portion approximately 48 hours post inoculation, then descended to the small intestine. L_3 moulted and converted into fourth-stage larvae (L_4) in the upper small intestine within 18 hours after the arrival. Subsequently the L_4 moulted to the young adults within 18 hours after the previous moult. The migration L_3 recovered from the cranial cavity, naso-frontal portion and small intestine differed from free-living infective L_3 in the following points: longer body size, larger esophagus ratio to body length, larger genital primordium, and morphological change of head as round or bulb-like. The body length of adult worms increased until 8 days post inoculation, and thereafter it gradually reduced together with the atrophy of the reproductive system.

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ラットにおける *Strongyloides ratti* の発育および形態学的変化

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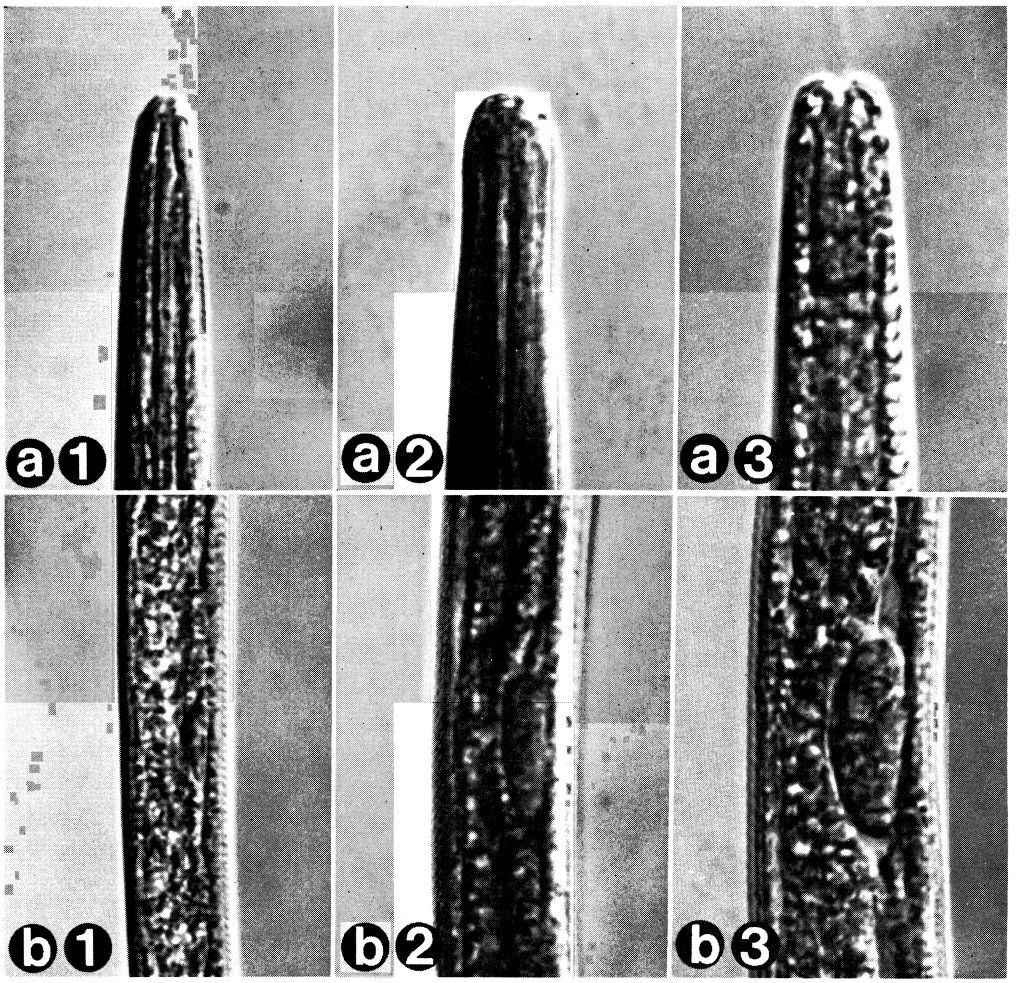
Strongyloides ratti はラットにおいて，従来の肺移行の知見に反し，頭部を移行するという阿部(1964)の報告が，近年，服部ら(1968)，多田ら(1979)によって定量的に確定された。今回，頭部移行を行なう *S. ratti* の形能学的変化を調べた．大腿内側皮下に接種された感染仔虫（L_3）は，48時間後に頭蓋腔・副鼻腔を通過後，咽頭を下降し小腸上部へ到達した．その後 L_3 は約18時間で脱皮し L_4 となり，さらに約18時間後に２度目の脱皮を行ない成虫となった．移行期の L_3 は接種前の L_3 と比較して，体長，食道比および生殖原基のサイズが増大していた．L_3 の頭端は，頭部へ移行した時点で，球状となった．小腸から回収された L_3 は表皮下に顕著な小顆粒を保有するものが認められた．成虫の体長は，接種後８日目まで増加したが，それ以降は漸次減少した．成虫の食道長は，ほとんど変化を示さなかったが，生殖器のサイズと生殖器内虫卵数の増減は体長の変化と平行していた．



Explanation of Figures

Fig. 2 Photographs of *Strongyloides ratti* third-stage larvae (L_3) recovered from various portions in rats, by Nomarski differential interference contrast microscope. ×1,000.
a, head; b, genital primordium; 1, before inoculation; 2, recovered from the nasofrontal portion (48 hours p. i.); 3, recovered from the small intestine (48 hours p. i.).