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Migration Route of *Strongyloides ratti* in Albino Rats

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Abe and his colleagues (1964, 1965a, b, 1966) investigated the migration route of *Strongyloides ratti* in rats. They concluded that infective larvae, when inoculated subcutaneously, migrated through muscle stroma to the head, lungs and finally reached the small intestine. They also showed the irreversibility of this route of migration. This finding differed from the known life cycle of *S. ratti* reported by previous workers, as the importance of the passage through the head was stressed. However, these findings of Abe *et al.* were mentioned only in a series of brief abstracts from meetings and were, for this reason, missing quantitative viewpoint. Hattori and others including one of us (Hattori *et al.*, 1968) and Hattori (1977) confirmed quantitatively the migration route proposed by Abe *et al.*

In the present study, we examined whether the same phenomenon would occur using *S. ratti* obtained from geographically different source. Special emphasis was laid on the quantitative study of larval distribution, the required time en route and the probable route of migration. We deal with a new migration route which differs from that of previous reports.

Materials and Methods

The strain of *S. ratti* used in this study was kindly provided by the Department of Medical Zoology, Tokyo Medical and Dental University and maintained in the authors' laboratory by successive passage in rats for more than 3 years. To confirm the identity of the strain, measurements and morphological observations were made and the results coincided with the data reported by Sandground (1925) and Little (1966).

Filariform larvae were harvested from filter paper cultures after 4 days of incubation at 27C. Five hundred filariform larvae, suspended in about 0.5 ml of physiological saline, were used for the infection of each rat.

Wister male rats which weighed approximately 180–190 g were used in each experiment. Subcutaneous inoculation of larvae was made on the abdominal side of the right femur and in Experiment I, intravenous inoculation was also made through the right femoral vein after incision of the skin. The rats were bled at 5–10 hour intervals by cardiac puncture after ether anesthesia.

The following organs and tissues were examined: head (cranial cavity, naso-frontal portion and brain), lungs, stomach, small intestine, liver, kidneys, spleen, blood from heart, muscles and various portions of skin. Reference to the cranial cavity means the combined washings of the surface of the inner cranium and the brain. The naso-frontal portion of the head is wedge-shaped and was separated from the bottom of cranium, mandible and the surrounding muscles, so

that it contained the nasal cavity and the major portion of the maxilla. The blood taken by cardiac puncture was immediately hemolyzed in distilled water and the sediment was examined. In Experiment I, the skin of the abdomen which included the inoculation site was removed in strips 3 cm long by 5 cm wide. In Experiments II and III, all body hair of the rats was removed before autopsy by using an electric hair clipper. After bleeding, 4 parts of the skin were removed separately ; lower portion (skin of lower trunk between tail and anterior edge of femur, but not including the legs), middle portion (skin between the lower and cervical portion), cervical portion (skin around neck between edge of ear and posterior edge of upper extremity) and cranial portion (all the skin of the head except the mandible). The individual portions of skin removed were not teased, but were placed separately in petri dishes on distilled water with the inner skin surface down for incubation. The superficial muscles taken from cervical, dorsal, femoral, lumbar, and abdominal areas were combined, minced and incubated together. Other parenchymous organs and tissues separated were teased by forceps, scissors and if bones are involved by cutting pliers into fine pieces for about one minute and incubated in petri dish with distilled water at 37C for about 100 minutes. The small intestine was split longitudinally and cut transversely into small segments in order to promote the emergence of parasites. All the incubated samples were examined under a compound microscope at 15 X—20 X magnification.

The number of ecchymoses present on the surface of the entire lungs were counted macroscopically. All rats were starved 12 hours before autopsy to avoid having food residue in the small intestine.

In the Experiment III, which was run parallel to Experiment II, the blockage of the migration of larvae was attempted in the neck region of the infected rats. The skin around the neck of 9 infected rats was cut transversely into the form of a collar and individual edges of skin were surgically sewn onto underlying muscle and fascia 5 mm apart from each other. White vaseline was then applied to the groove-like exposed muscle layer in order to prevent from the fusion of separated skins. This group of rats was sacrificed at 20, 30 and 50 hours p. i.

In order to observe histologically the migrating larvae in the tissues, cervical skin was removed from rats at 20 hours p. i. and was fixed in 10% formalin. The sectioned tissues were stained with hematoxylin and eosin. The naso-frontal portion of the head was decalcified with 5% nitric acid for 3 days after the fixation with 10% formalin, and was stained by the same procedure.

In the Experiment I, the study was designed to check the migration of larvae from inoculation site to the head region using the above mentioned strain of *S. ratti* according to our previous method of examination (Hattori *et al.*, 1968). The second purpose of this experiment was to compare the subcutaneous inoculation with the intravenous one chronologically. The purpose of Experiment II was to know the role of the route through skin and the underlying tissue in the migration of filariform larvae between inoculation site and the head. The Experiment III aimed at the confirmation of subcutaneous and cutaneous migration of the larvae by the surgical blockage around the neck.

Results

1. *Experiment I.*

The identical lot of larvae harvested was inoculated into 2 groups of rats through either subcutaneous or intravenous routes. The chronological examinations were made quantitatively (Tables 1 and 2). As no larvae were recovered from blood, liver, spleen and kidneys at any autopsy times in both series of inoculations, these columns are omitted from the tables.

1) Subcutaneous inoculation. A very small number of larvae were found in the inoculation site at 10–15 hours post inoculation

Table 1 Distribution of larvae of *S. ratti* subcutaneously inoculated into rats per elapsed time (500 filariform larvae per rat)

Autopsy time (hr.)	No. rats examined	Average No. ecchymoses on lungs	Total No. larvae recovered (%)	No. larvae recovered						
				Head			Lungs	Stomach	Small intestine	Inocul. site
				Cranial cavity	Brain	Nasofrontal portion				
10	2	12.5	6.5(1.3)	0	0	0	0	0	0	6.5
15	2	31.0	5.5(1.1)	0	0	0	2.0	0	0	3.5
20	2	7.5	27.0(5.4)	20.5	2.0	4.0	0.5	0	0	0
25	2	0.5	25.0(5.0)	19.5	1.5	2.0	1.0	0	0	1.0
30	2	37.5	93.0(18.6)	64.5	1.0	27.0	0.5	0	0	0
40	2	4.5	195.0(39.0)	19.0	1.5	171.5	0	0	3.0	0
50	2	7.5	42.0(8.4)	3.0	0.5	25.0	0.5	2.0	10.5	0

Table 2 Distribution of larvae of *S. ratti* intravenously inoculated into rats per elapsed time (500 filariform larvae per rat)

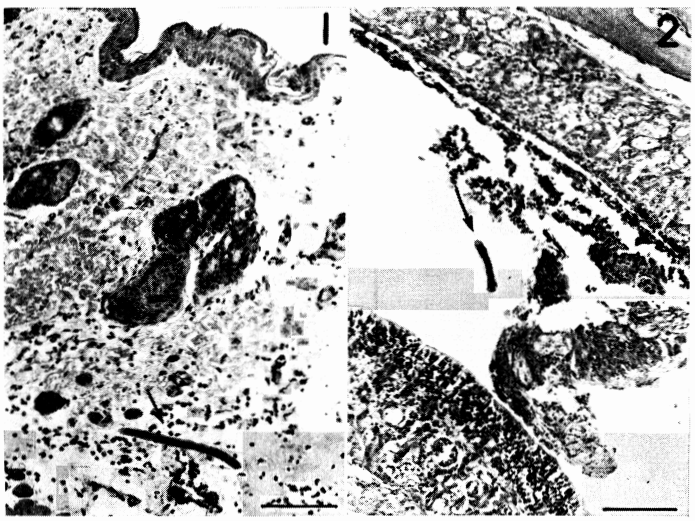
Autopsy time (hr.)	No. rats examined	Average No. ecchymoses on lungs	Total No. larvae recovered (%)	No. larvae recovered					
				Head			Lungs	Stomach	Small intestine
				Cranial cavity	Brain	Nasofrontal portion			
10	2	230.0	54.5(10.9)	0	0	0	54.5	0	0
15	2	207.5	86.0(17.2)	0	0	0	86.0	0	0
20	2	215.0	109.0(21.8)	0	0	0.5	108.5	0	0
25	2	215.0	89.5(17.9)	4.0	0	1.5	84.0	0	0
30	2	190.0	95.0(19.0)	5.5	0	2.0	87.5	0	0
40	2	156.5	63.5(12.7)	5.5	0	13.0	21.0	0.5	23.5
50	2	86.5	20.5(4.1)	0.5	0.5	10.0	0.5	0	9.0

(p. i.). At 20 hours, the larvae had already migrated to the head, especially into the cranial cavity and the number of larvae in this location increased with time until 40 hours p. i. From 40 hours p. i., larvae began to appear in the small intestine and tended to increase there. At this time, the number of larvae was markedly reduced in the head region. Very few parasites were recovered from the lungs.

2) Intravenous inoculation. There was a striking change in the appearance of lungs through all the autopsies. About 200 or more ecchymoses were usually found on the surface of lungs during the period between 10 and 30 hours p. i. Later, the ecchymoses were reduced in number and in color. Almost all the larvae recovered were from lungs until 30 hours p. i., while virtually all of the larvae were seen in the head of rats infected subcutaneously. Some larvae started to appear in the head portion although there was 5–10 hour delay in comparison with that seen in subcutaneous inoculation. Regardless of the way of inoculation, parasites reached the small intestine 40–50 hours p. i. and began postfilariform growth immediately. It should be noted that the subcutaneous inoculation of larvae resulted in very low recoveries of larvae from the lungs in comparison with the intravenous inoculation.

2. *Experiment II.*

In this experiment, blood, spleen, kidneys and liver were not examined, because of the size of the inoculum on the basis of the results from Experiment I. Instead, all the skins of infected rats were intensively examined. The general features of migration were similar to the results seen in the preliminary experiment. In the initial stage of inoculation, the larvae were found exclusively in the skin and its underlying connective tissue (Photo. 1), then the larvae appeared in the head at 15 hours p. i. (Table 3 and Fig. 1). They moved to the small intestine at 40 hours p. i. or later. The filariform larvae showed rapid growth to become parasitic females in the small intestine. Muscles, so far as examined, seemed not to play an important role in the migra-



Explanations of Photographs

Photo. 1 A portion of *S. ratti* larva (arrow) migrating in the lower part of cutis of cervical skin 20 hours post inoculation (scale, 100 μ).

Photo. 2 A portion of *S. ratti* larva (arrow) migrating in the space between septum and concha nasalis of an infected rat 40 hours post inoculation (scale, 100 μ).

Table 3 Distribution of inoculated larvae of *S. ratti* in rats arranged by the time elapsed (Experiment II). 500 filariform larvae were subcutaneously inoculated

Autopsy time (hr.)	No. rat examined	Average No. ecchymoses on lungs	Total No. larvae recovered (%)	No. larvae recovered*									
				Skin				Muscle	Head			Lungs	Small intestine
				Lower portion	Middle portion	Cervical portion	Cranial portion		Cranial cavity	Brain	Nasofrontal portion		
5	4	8.8	12.2(2.4)	8.3	3.0	0.3	0	0.3	0	0	0	0.3	0
10	4	4.5	97.6(19.5)	79.5	11.0	2.3	0	3.5	0	0	0	1.3	0
15	4	4.3	60.8(12.2)	3.3	15.3	14.8	7.5	3.8	11.0	0.3	3.0	1.8	0
20	4	2.3	104.2(20.8)	0	0.8	6.3	5.5	0	50.5	0.5	40.3	0.3	0
25	4	12.5	151.5(30.3)	0.5	0.3	2.8	1.3	0	44.5	8.8	91.3	2.0	0
30	4	26.8	221.1(44.2)	0	0	0.5	0	0	40.3	9.5	158.8	12.0	0
40	4	20.0	217.6(43.5)	0	0.3	0	0	0	59.5	2.0	29.5	0.5	125.8**
50	4	9.0	226.3(45.3)	0	0	0	0	0	4.0	9.5	172.5	0.3	40.0
60	4	16.3	226.1(45.2)	0	0	0	0	0	2.3	0.3	2.0	0	221.5
70	4	1.5	55.0(11.0)	0	0	0	0	0	0	0	0	0	55.0

* Average number of larvae obtained from the rats examined.
** The data include postfilariform larvae or young adults.

Table 4 Comparison of the distribution of inoculated larvae between the rats with dermal incision and the controls

Autopsy time (hr.)	No. rat examined	Animal group	Total No. larvae recovered	Percentage of larvae recovered									
				Skin				Muscle	Head			Lungs	Small intestine
				Lower portion	Middle portion	Cervical portion	Cranial portion		Cranial cavity	Brain	Nasofrontal portion		
20	4	control	104.2*	0.0	0.8	6.0	5.3	0.0	48.5	0.5	38.7	0.3	0.0
	3	incision	84.0	1.5	4.8	88.1	2.7	1.2	0.4	0.0	0.4	0.8	0.0
30	4	control	221.1	0.0	0.0	0.2	0.0	0.0	18.2	4.3	71.8	5.4	0.0
	3	incision	59.3	0.5	2.2	64.1	1.7	1.2	19.1	0.0	10.6	0.5	0.0
50	4	control	226.3	0.0	0.0	0.0	0.0	0.0	1.8	4.2	76.2	0.1	17.7
	3	incision	94.0	0.0	0.3	16.7	0.7	0.0	12.4	1.4	66.0	0.3	2.1

* Average of total number of larvae recovered from 4 infected rats shown in Table 3.

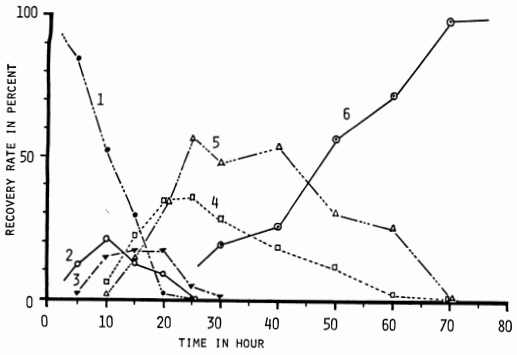


Fig. 1 Recovery of inoculated larvae from the organs and tissues of albino rats experimentally infected. Showing the change of hourly recovery rate of larvae in various portions of body as a percentage of the total number of larvae found at each time. The curves were obtained by the triple running average. 1, lower portion of skin; 2, middle portion of skin; 3, cranial and cervical portion of skin; 4, cranial cavity and brain; 5, naso-frontal portion of head; 6, small intestine.

tion, because only a very few larvae were found in the early stage of infection. On the other hand, the skin and its connective tissues contained considerable numbers of larvae in the initial stages (5–15 hours p. i.) of inoculation. The minimum rate of recovery of all the inoculated larvae was 2.4% at 5 hours p. i. and the maximum, 45.3%, at 50 hours p. i. in this experiment.

The hourly percentages of recovered larvae from individual portions are shown in the Fig. 1. In this figure, triple-running averages were calculated due to the data shown in Table 3 for each point, in order to get smoother curves conforming to the main trend. The figure gives a clear outline of the migration of *S. ratti* larvae in the body of rats. Most of the larvae were recovered from the lower portion of skin at 5 hours p. i., and the larvae moved gradually to the upper portion of the skin with time. The ascending migration of larvae was considerably rapid, since they appeared in the cranial cavity and naso-frontal portion even by 15 hours p. i. (Photo. 2). However, the actual time for the beginning and end points of larval appearance may be obscure in this figure, because of the special way of calculation.

3. *Experiment III.*

Three groups of 3 rats each were infected subcutaneously and the cervical skin was separated surgically as mentioned previously. The distribution of larvae from each rat group at 20, 30 and 50 hours p. i. is shown in Table 4, together with that of controls which were simultaneously sacrificed in Experiment II. In the rats surgically manipulated, a large proportion of the larvae were found in the peripheral portion of cervical skin for a long period until 30 hours p. i., while only a negligible percentage of larvae was found in the same region in control rats. This finding suggested that larvae had difficulty in migrating over the skin cleavage. By 50 hours p. i., the general pattern of larval distribution was similar in both experiments. However, there is also an apparent delay in the migration of larvae in the surgically treated rats. This finding suggests that the skin and its underlying connective tissue play a very important role in the ascending migration route of *S. ratti* larvae between the penetration site and the head of rats.

Discussion

Previous workers have examined the mode of infection of *S. ratti* in the rat host and described the life cycle in which larvae penetrated the skin and passed to the lungs, trachea, esophagus and finally reached to small intestine. The migration through the lung has been stressed. In experimental infections, however, large numbers of larvae were usually inoculated and the larvae then showed a random dispersion to various parts of the body. Abadie (1963) followed the life cycle and development of *S. ratti* by infecting rats immersing the tail in the inoculum which contained from 1,000 to 10,000 larvae and examined their distribution in the body. He failed to find larvae in the brain, eyes, kidneys, pancreas, heart and peritoneal cavity, but did demonstrate them in blood, liver,

lungs and small intestine. In a series of preliminary experiments by Abe *et al.* (1964, 1965 a, b, 1966), they provided evidence for a different route of migration as follows: subcutaneous tissue—stroma in the muscle of whole trunk—cranial muscle—nasal cavity—cranium—brain—(via blood stream)—lungs—trachea—esophagus—small intestine. Later, Hattori *et al.* (1968) and Hattori (1977) carried out experiments of similar design quantitatively and were able to confirm this route. However, they could not reach a final conclusion as to the route of migration between the inoclation site of subcutis and cranium, and further, they proposed a question with regard to the importance of lungs.

In the present study, we infected rats with relatively lower doses of 500 filariform larvae, in order to prevent possible random dispersion. This experiment has confirmed the original observations by Abe *et al.* (1964) using a different strain. Our chronological study also roughly confirmed the previous results of Hattori and others. Based on our chronological and quantitative studies, it will be concluded that the larvae of *S. ratti* must undergo a cranial migration before they finally migrate to the small intestine. However, there are still some questions concerning the significance of larval migration to the head. In regard to the mode of entry of larvae, there is certainly no difference between subcutaneous and percutaneous infection in relation to the passage of larvae to the head. In a preliminary experiment, 1,200 larvae were percutaneously administered through abdominal skin of anesthesized rat and 50 larvae were recovered from the head 28 hours later. The similar result was also previously reported by Abe *et al.* (1965 a). Based on these findings, it may be recommended to use subcutaneous inoculation in order to obtain quantitative result.

It was noticed that in the initial stage of infection, 5–10 hours p. i., a great majority of the recovered larvae were from the skin and the underlying connective tissue (Experiment II). When a blockage of skin around neck region was made on the infected rats, the migration of larvae was markedly delayed. There was a concentration of migrating larvae on the peripheral side of the cervical skin in comparison with controls. However, the effect of dermal incision was temporary, because at 50 hours p. i., about 80% of recovered larvae were from the head. This finding demonstrates the presence of compensatory migration routes for these larvae, probably the connective tissues and spaces among muscle bundles of neck region.

The low recovery rate of larvae shown in the incision rats (Table 4) leads us to a hypothesis that the dermal blockage caused disturbance of migration and the consecutive dispersion of larvae. On the other hand, only a few parasites were recovered from body muscles during 5–15 hours p. i., and at no other autopsy times. This finding differed from that of previous reports by Abe *et al.*. A further investigation is needed to evaluate the role of muscle stroma in the course of larval migration.

Wertheim and Lengy (1965) recognized that larvae found in the lungs were somewhat longer than infective ones and have undergone internal changes indicative of growth. Abe *et al.* (1966) considered that the larvae migrated from the brain to the lungs via blood vessels and thought that the lung also played an important role. As shown in Table 3, there were only a few numbers of larvae in the lung between 5 to 50 hours p. i., and the decrease of larvae in the head was followed rapidly by an increase of larvae in the small intestine. This finding indicates the less important role of the lung during the migration of *S. ratti* larvae.

The inoculation of larvae into the femoral vein demonstrated many ecchymoses on the surface of the lungs and represented the extravasation of larvae (Table 2). Almost all of the recovered larvae were still in the lungs until 30 hours p. i. without further migration. This finding shows the different behavior of the larvae when inoculated through the circulatory system, in comparison with those inoculated subcutaneously. Thereafter, one-third of the larvae were

recovered from the lungs, one-third, from the head and the rest, in the small intestine, respectively. However, it is not known whether larvae migrated directly to the digestive tract or indirectly to there via the head in this experiment. It should be considered that generally the larvae migrate to the head rather through various loose spaces especially the subcutis, than through circulatory system.

With regard to the recovery rate of inoculated larvae at determined times from quantitative view, the only comparable data are of Hattori *et al.* (1968) and Hattori (1977). Our present result (Table 3) coincided with the recovery rate of 5.3–41.2% mentioned by the above workers. In order to recover more larvae and to clarify the location of undetected parasites in the infected animals, technical procedures need to be improved. However, the present experimental design presumably reflects the outline of the migration route of *S. ratti* larvae in albino rats.

Summary

The migration route of *Strongyloides ratti* larvae was studied chronologically and quantitatively in albino rats infected either by subcutaneous or intravenous routes with 500 filariform larvae. The larvae migrated through the loose subcutis to the cranial cavity and naso-frontal portion of the head by 15–50 hours post inoculation (p. i.) and then migrated to the small intestine 40 hours p. i. and later. The present study confirms previous observation on the invasion of larvae into cranium using a different strain of *S. ratti* and demonstrated the presence of a subcutaneous migration route between the inoculation site and the head for the first time.

Acknowledgements

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ラットにおける *Strongyloides ratti* 幼虫の移行経路

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Strongyloides ratti 感染型幼虫は，ラット皮下に注入すると，頭部を通過し，最終的に小腸に達することを阿部ら（1964，1965a，b，1966）が見出した．この現象については，服部ら（1968），服部（1977）によつて定量的な裏付けが為された．今回の実験では，異つた株の *S. ratti*（東京医科歯科大学医動物学教室で維持していたものを3年余当教室で継代維持）を少数（500 L_3）皮下あるいは静脈内に投与し，経時的に夫々のラット群を剖検した．皮下注入された幼虫は注入15～50時間後に頭部に集中し，その後徐々に下行し小腸で発育を始めた．この所見は上述の阿部らの認めた現象が，異つた株を用いても，あるいは少数の L_3 を使つても本質的な現象として見られることを示している．次に大腿静脈内に感染幼虫を注入した場合，幼虫は25～30時間後まで主として肺に滞在し，その後皮下注入群に比べ10時間ほどおくれて，幼虫の一部は頭部へ移行し，一部はすでに小腸に見られた．大腿内側の皮下注入部位から頭部への移行は，阿部らの言う“筋肉間質”経由でなく，むしろ皮下通過に特徴があると考えられた．この事実は，頸部皮膚をカラー状に切離して筋肉部に縫いつけ，露出した部分にワセリン塗布したラットにおいては頭部移行が著明に抑制されることからも裏付けられた．