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The occurrence of *Strongyloides ratti* in the tissues of mice after percutaneous infection

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ABSTRACT

The migration of infective larvae of *Strongyloides ratti* has been examined in C57B1/6 mice after percutaneous infection of the anterior abdominal wall. Lateral migration of larvae through the skin and subcutaneous tissues was not seen. Large numbers of larvae were recovered from the muscles between 2 and 24 hours after infection and larvae were seen in the cerebrospinal fluid 24 and 48 hours after infection. Insignificant numbers of larvae were seen in the blood, serosal cavities, liver, spleen, kidneys, brain or nasopharynx. Larvae arrived in the lungs between 24 and 72 hours after infection and worms were first noted in the small intestines at 48 hours. It is concluded that larvae migrate preferentially to the muscles and CSF before passing to the lungs, but the exact mode of travel is uncertain.

INTRODUCTION

The life-cycle of *Strongyloides ratti* in rats was described in a qualitative fashion by ABADIE (1963) and WERTHEIM & LENGY (1965). Both of these authors appear to have assumed that larvae followed the classical pathway from the skin via the bloodstream to the lungs and thence to the small intestine. More recently, TADA *et al.* (1979) have examined the route of migration of *S. ratti* in rats in more detail. They concluded that the larvae migrated through the subcutaneous tissue to the cranial cavity and the nasofrontal portion of the head and then via an ill-defined route to the intestines.

We have recently shown that infections with *S. ratti* develop in C57B1/6 mice (DAWKINS *et al.*, 1980). We then examined the kinetics of infection by quantifying larvae in the skin, lungs and small intestines of mice during primary and secondary infections as these tissues had seemed to be of major importance (DAWKINS & GROVE, 1981). In the light of the observations by TADA *et al.* (1979), however, we have re-examined this system in more detail in order to ascertain whether *S. ratti* follows a pattern of migration in mice, similar to that described by those authors in rats.

MATERIALS AND METHODS

Female C57B1/6 mice 18–22 g in weight, supplied by the Animal Breeding Unit, University of Western Australia (Perth W.A.) were used throughout the study.

An homogonic strain of *Strongyloides ratti* has been maintained by serial passage in Sprague-Dawley rats as described previously (DAWKINS *et al.*, 1980).

Mice were anaesthetized with sodium pentobarbitone, then the abdomen shaved over an area of approximately 15 × 15 mm and the skin dampened with water. In order to facilitate infection, mice were immobilized with adhesive tape for 20 min during which time percutaneous (p.c.) infection was achieved by applying filariform larvae in 50 μl of phosphate buffered saline (PBS) to the skin. After infection, but before the mice were released, the site of infection was washed thoroughly with water to remove any larvae which had not penetrated the skin.

35 mice were infected with 2,500 larvae p.c. 50 μl samples of blood were taken from the retro-orbital venous plexus, 5, 10, 15, 30, 60 and 180 minutes after infection. Five animals were killed two, six, 12, 24, 48, 72 and 96 hours after infection. In each case the entire ventral surface of the mouse was shaved in order that a section of thoracic skin,

15 × 15 mm, and the anterior abdominal wall approximately 20 × 20 mm could be removed relatively free of fur. The pectoral muscles and the hamstring muscles were excised, weighed and treated separately. The peritoneal and thoracic cavities were irrigated with PBS and the washings collected. The lungs, with the heart intact, were removed as was the small intestine. The brain was taken out of the cranial cavity. The fluid within the cranial cavity was aspirated. Both the surface of the brain and the cranial cavity were gently washed with PBS to collect any remaining larvae. These washings were added to the fluid aspirated from the cranial cavity and henceforth are referred to as cerebrospinal fluid (CSF). The skin over the face was stripped off, then the naso-maxillary region was excised after separation of this area from the base of the cranium, the mandible and the surrounding muscles. The liver, spleen and kidneys were removed.

The anterior abdominal wall, thoracic skin, pectoral muscles, hamstring muscles, heart and lungs, liver, spleen, kidneys, small intestine, brain and the naso-maxillary region were homogenized in a Waring blender at high speed for 15 seconds. The tissue homogenates were suspended in 10 to 25 ml of PBS and the larvae in multiple (2 to 10) 0·5 ml samples were counted. The CSF and serosal washings were centrifuged at 300 *g* for 5 min and the number of larvae in the pellet counted.

RESULTS

Anterior abdominal wall: Peak numbers of the larvae were seen 2 hours after infection, with 26% of the larvae that were applied being recovered (Fig. 1). No significant change occurred over the ensuing 10 hours but larval numbers declined rapidly thereafter with small numbers being found at 24 hours ($P<0{\cdot}001$). A few larvae were observed to persist over the next three days.

Thoracic skin: Very few larvae were ever recovered from the thoracic skin. (Fig. 1).

Blood: no larvae were ever seen.

Muscles: Larvae were recovered from the pectoral and hamstring muscles between 2 and 24 hours after infection (Fig. 2). There were no significant differences in the

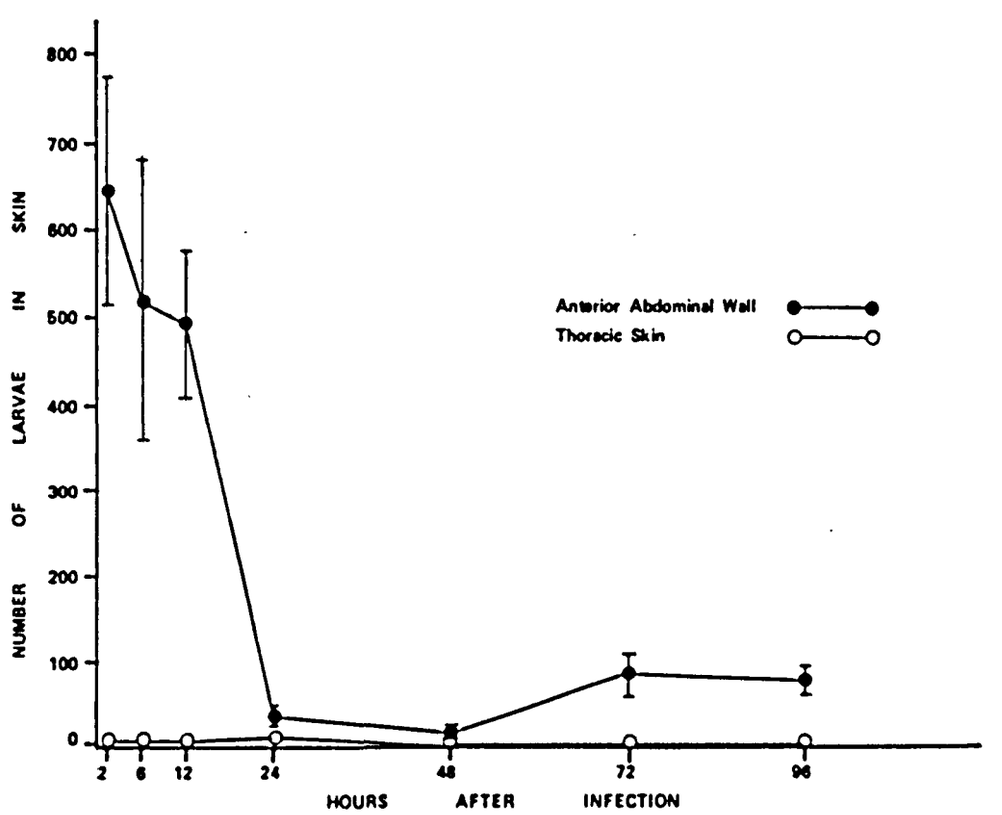

FIG. 1. Larval numbers recovered from the anterior abdominal wall (●—●) and the thoracic skin (○—○) at varying times after infection. Results are expressed as the mean ± S.E.

numbers of larvae recovered from the two muscle groups at 2, 6, 12 and 24 hours. Peak numbers of larvae were seen in the muscles between 12 and 24 hours.

CSF: Larvae were first seen in the CSF 24 hours after infection (Fig. 3). Peak numbers were noted 48 hours after infection. Small numbers were found 3 and 4 days after infection.

Brain: Very few larvae were seen in the brain (Table I).

Nasopharynx: Very few larvae were seen in the naso-maxillary region (Table I).

Heart and Lungs: A few larvae were noted 2 hours after infection but none were seen when these tissues were examined at 6 and 12 hours (Fig. 4). Larvae reappeared 24 hours after infection and peak numbers (2·4% of those applied) were seen 48 hours after infection. Small numbers of larvae were recovered 72 and 96 hours after infection.

Liver, spleen, kidneys, peritoneal and thoracic cavities: Very few larvae were ever seen (Table 1).

Small intestine: Small numbers of larvae were first seen in the small intestine 48 hours after infection. Significantly more worms ($P<0·02$) were noted 3 and 4 days after infection.

DISCUSSION

In this paper we have confirmed our previous observations (Dawkins & Grove, 1981) on the kinetics of infection in the skin, lungs and the small intestine. We have attempted to define the events which occur between penetration of the skin by larvae and the departure of larvae from the lungs. In contrast to the observations of Tada *et al.* (1979), we could find no evidence of lateral migration of filariform larvae through the

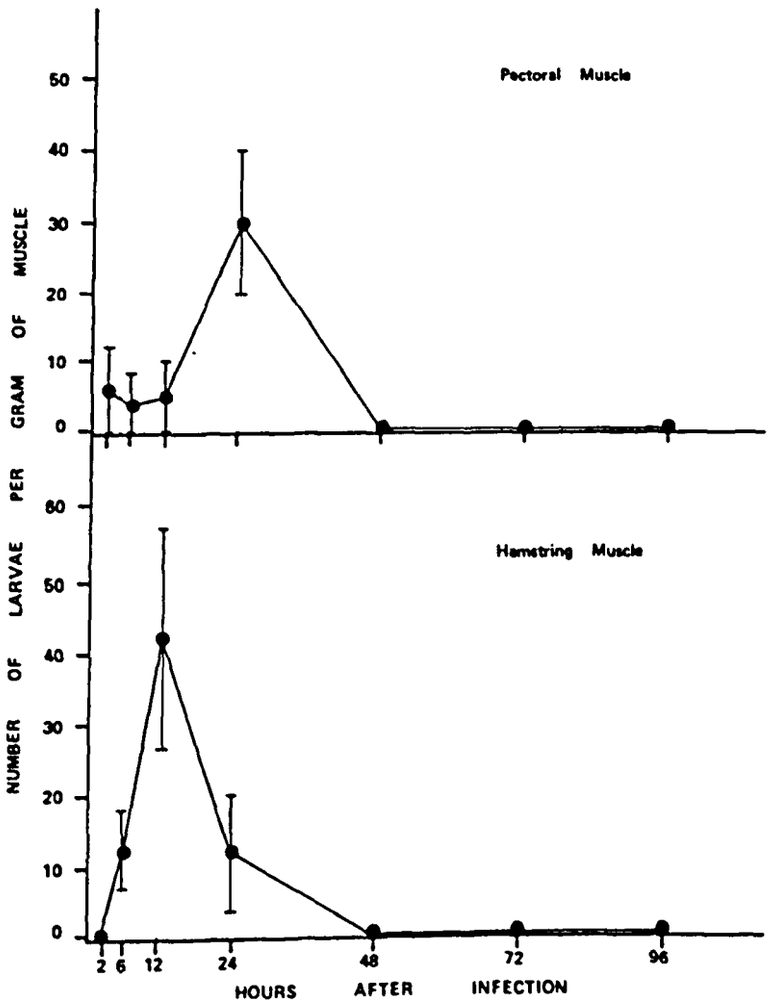

FIG. 2. The number of larvae per gram of skeletal muscle at various times after infection with *S. ratti*. Results are expressed as larvae per gram of muscle ± S.E.

subcutaneous tissues adjacent to the site of infection. This may be because we have used a different strain of *S. ratti* and a different host species from that of TADA and co-workers.

According to TADA *et al.* (1979), ABE *et al.* (1965a, 1965b) and HATTORI *et al.* (1968) considered that larvae migrated through the muscles to the head and lungs. We also found substantial numbers of larvae in the muscles. Peak numbers were noted between 12 and 24 hours after infection. The concentrations of larvae in the muscles at different sites (pectoral and hamstring muscles) were similar. If the muscle mass is considered to constitute 40% of the weight of a mouse, then approximately 340 larvae (13% of those

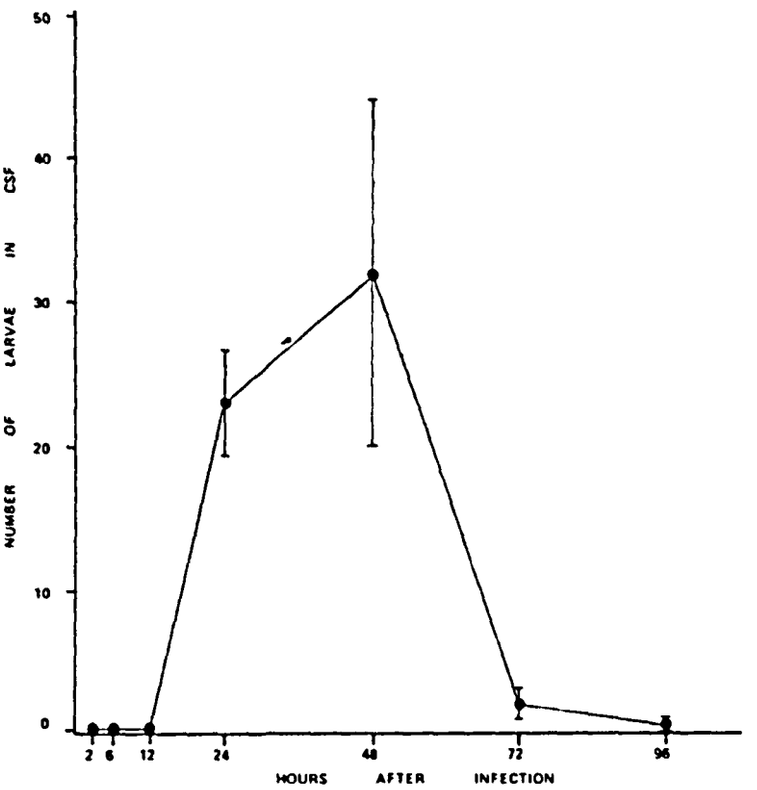

FIG. 3. Larval numbers in the cerebrospinal fluid at various times after infection. The results are expressed as the mean ± S.E.

TABLE I

Larvae recovered from brain, nasopharynx, liver, spleen, kidneys, peritoneal and thoracic cavities and small intestine at intervals after infection. The results are expressed at mean ± S.E. per organ

Hours after infection	Brain	Nasopharynx	Liver	Spleen
2	0	0	N.D.	N.D.
6	0	0	N.D.	N.D.
12	0	0	N.D.	N.D.
24	3±3	2±2	0	0·3±0·3
48	0	4±4	0	0
72	8±5	2±2	0	0
96	0	11±5	N.D.	N.D.

	Kidneys	Peritoneal cavity	Thoracic cavity	Small intestine
24	0	0·3±0·3	0	0
48	0	0·7±0·7	0	27±6
72	0	0	0	112±28
96	N.D.	N.D.	N.D.	114±31

N.D. = not done
S.E. = standard error of mean

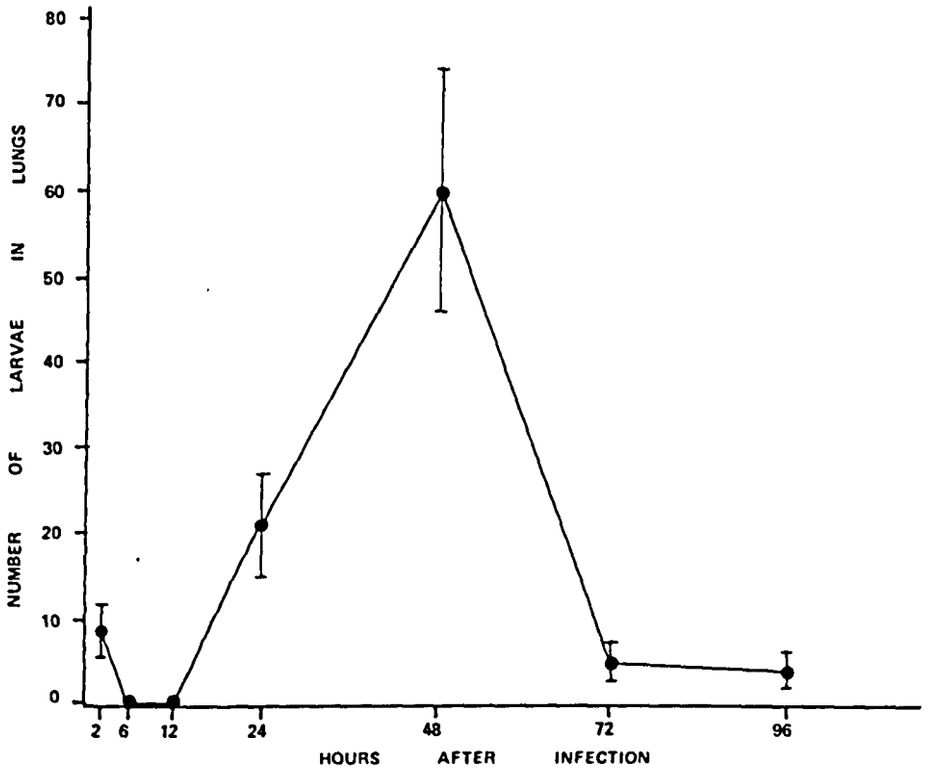

FIG. 4. Larval numbers in the lungs of mice at certain times after infection. The results are expressed as the mean ± S.E.

applied) could be accounted for in the skeletal muscles. Nevertheless, the exact means by which the larvae reached the muscles from the skin of the anterior abdominal wall is still obscure; they could have migrated either via the bloodstream or directly through the deep connective tissues and musculature.

Soon after larvae were noted in the muscles, worms were found in the cerebrospinal fluid, although in relatively small numbers. Larvae were first seen 24 hours after infection and they persisted for one to two days. Thus, we have confirmed the observations of TADA *et al.* (1979) who detected larvae in the CSF of rats between 15 and 40 hours after infection. The cerebrospinal fluid may be a preferred location for filariform larvae as it provides a liquid-filled space allowing ease of movement and plentiful nutrients. We found a few larvae in homogenized brain; they may have been located in the CSF of the ventricles.

Neither ABADIE (1963) nor TADA *et al.* (1979) found significant numbers of larvae in visceral organs other than the lungs and gut. On the other hand, SPINDLER (1958) recovered larvae from the heart, liver and gonads as well as the muscles, after infection of rats with large numbers of larvae (50,000). On a weight for weight basis, the intensity of infection in our mice was of the same order as that given to rats by SPINDLER (1958). Our failure to demonstrate significant numbers of larvae in the liver, spleen or kidneys, despite their abundant blood supply, suggests that there is a specific tropism of filariform larvae for the muscles and CSF.

TADA *et al.* (1979) found large numbers of filariform larvae in the nasofrontal region of the rats. We were unable to duplicate this finding. We found a few larvae in this region between 24 and 96 hours after infection. These larvae may have been migrating from the lungs through the pharynx to the intestines and inadvertently found their way into the nasopharynx and the nasal passages.

Larvae tended to appear in the lungs 12 to 24 hours after they were seen in muscles and CSF. Again, the route of migration from these tissues to the lungs is uncertain. They may have passed via the blood stream or migrated directly through the connective tissues, although this latter possibility seems less likely. As has been demonstrated

previously, larvae passed from the lungs to the gut where they matured (DAWKINS & GROVE, 1981).

In conclusion, we have shown that in mice, *S. ratti* larvae migrate preferentially to the muscles and are found in the CSF confirming the observations of ABE *et al.* (1965a, 1965b) HATTORI *et al.* (1968) and TADA *et al.* (1979). We were unable to demonstrate lateral migration of the larvae through the skin and subcutaneous tissues and we were not able to confirm a high concentration of larvae in the nasopharyngeal region. The exact mode of travel through the body is still uncertain. It is possible that histological studies may differentiate between passage of larvae in the blood stream and direct migration through the connective tissues.

ACKNOWLEDGEMENTS

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