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KINETICS OF PRIMARY AND SECONDARY INFECTIONS WITH *STRONGYLOIDES RATTI* IN MICE

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Abstract—DAWKINS H. J. S. and GROVE D. I. 1981 Kinetics of primary and secondary infections with *Strongyloides ratti* in mice. *International Journal for Parasitology* **11**: 89–96. The kinetics of infection with *S. ratti* were quantitated in normal and previously exposed C57Bl/6 mice. In primary infections, larvae penetrated the skin rapidly and were seen in peak numbers 12 h after infection. By 24 h after infection, larval numbers had declined appreciably and there was a slow decrease in numbers thereafter. Larvae were first observed in the lungs at 24 h and maximal recovery occurred at 48 h. It is thought that larval migration through the lungs is rapid. Worms were first seen in the intestines two days after infection. Maximum numbers were seen on the fifth day and worm expulsion was complete by day 10. Two moults took place in the small intestine during days 3 and 4 after infection. Rhabditiform larvae were first noted on the fourth day after infection. Mice exposed to *S. ratti* four weeks previously had significantly less larvae in the skin 4 and 12 h after infection but by 24 h there was no difference when compared with mice with primary infections. Peak recovery of larvae from the lungs occurred 24 h after infection; significantly less larvae were recovered on days 2 and 3 when compared with normal mice. There was a marked reduction in the adult worm burden in the gut; the number of worms recovered was less than one fifth of that seen in primary infections. Those worms which did mature were less fecund and were expelled from the intestines within 7 days of infection. It is suggested that in previously exposed animals, the migration of larvae from the skin is hastened, many of these larvae are destroyed in the lungs and that expulsion of worms which do mature in the intestines is accelerated.

INDEX KEY WORDS: *Strongyloides ratti*; C57Bl/6 mice; kinetics; larval migration, primary infection; secondary infection; worm rejection; fecundity; moult.

INTRODUCTION

THE LIFE cycle of *Strongyloides ratti* in rats has been described in a qualitative fashion by Abadie (1963) and Wertheim & Lengy (1965). However, any analysis of factors influencing the host–parasite relationship requires quantitative information concerning the kinetics of infection. Attempts have been made to quantify some aspects of infection (Moqbel & Denham, 1977; Olson & Schiller, 1978), but these have been limited by the technical difficulties of counting worms in large amounts of tissues. Indeed, Wilson (1979) stated that "heroic feats of necropsy involving direct counts of larvae are described in the relevant literature, but these tend to be uneconomic and possibly misleading in outcome" and went on to attempt to develop a method for tracking radioactive larvae of *S. ratti* in the tissues.

An alternative approach is to use an animal host which is smaller and thus more economical and easy to manipulate. We have recently shown that *S. ratti* will infect certain inbred strains of mice (Dawkins, Grove, Dunsmore & Mitchell, 1980). We have used this system to quantitate the course of primary and secondary infections in C57Bl/6 mice infected with *S. ratti*.

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MATERIAL AND METHODS

Animals. Female C57Bl/6 mice, 18–22 g in weight, and random-bred Sprague–Dawley rats, 150–200 g in weight, were supplied by the Animal Breeding Unit, University of W.A.

Strongyloides ratti. An homogonic strain of *S. ratti* has been maintained by serial passage in rats as described previously (Dawkins *et al.*, 1980). Mice were anaesthetised with sodium pentobarbitone, then the abdomen was shaved over an area of approx. 15 × 15 mm and the skin dampened with water. In order to facilitate infection, mice were immobilised 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. Ninety mice were infected p.c.

with 400 filariform larvae. Patent infections were confirmed seven days later by the demonstration of larvae in the faeces. Four weeks after the initial infection, 90 immune mice and 90 control littermates were infected p.c. at the same site with 3000 larvae. At various intervals after infection six mice from each group were killed by cervical dislocation and the organs removed. The shaved section of the anterior abdominal wall, 15 × 15 mm, was washed thoroughly with PBS in order to remove any larvae that had not penetrated, and the skin was then removed. This tissue was minced with scissors, homogenised in 40 ml PBS in a Waring blender at high speed for 15 s, then made up to 50 ml in centrifuge tubes and the numbers of larvae in multiple (4–10) 0·5 samples were counted. The lungs were removed with the heart intact, homogenised as described previously, and multiple 0·5 ml samples were counted. The small intestines were removed and homogenised for only 10 s then multiple 0·5 ml samples were counted. This technique gave satisfactory worm counts, but since some worms were damaged, larvae which were used for the measurement of worm length were obtained by removing the small intestines, slicing them longitudinally, then incubating them on a gauze mesh in PBS at 37°C overnight. The sedimented worms were collected and fixed in 10% buffered formalin solution at 40°C. Worm lengths were measured using a standardised graticule eyepiece (Graticules Ltd., England). All tests of statistical significance were made using Student's *t*-test.

RESULTS

Recovery of larvae from skin

The abdominal skins of control and immune mice were examined 2, 4, 12 and 24 h and 2, 3, 6 and 9 days after infection (Fig. 1). In control animals, the number of larvae recovered increased from 320 ± 74 (mean ± S.E.M.) at 2 h to a peak of 560 ± 73 at 12 h although this difference was not significant. The maximum percentage of larvae which was recovered from the skin was 18·5% of those applied. Over the next 12 h, larval numbers diminished significantly ($P < 0.001$). Thereafter, there was a slow decline in the numbers of larvae recovered with increasing duration of infection. Nine days after infection, no larvae were found.

In immune mice, the numbers of larvae were much lower than in control animals, with larval recoveries being significantly less at 4 h ($P < 0.025$) and 12 h ($P < 0.005$) after infection. The maximum percentage of larvae recovered from the skin was 6·1%, 2 h after infection. There was a slow decline in numbers with increasing duration of infection and no larvae were found in the skin after 6 days.

Recovery of larvae from lungs

The lungs of control and immune mice were examined 2, 4, 12 and 24 h and 2, 3, 4 and 9 days after infection (Fig. 2). In control animals, larvae were first recovered 24 h after infection. Peak numbers were seen after 48 h and no larvae were detected on days 6 and 9. The maximum percentage of worms recovered from the lungs at any one time was 2·4% of those applied.

In immune mice, larvae were first observed 24 h after infection. This was also the time of peak recovery, although it was not significantly different from that seen in control animals. The maximum number of larvae recovered was 1·4%. Significantly less larvae were recovered 2 and 3 days after infection when compared with normal mice ($P < 0.005$ and $P < 0.02$, respectively). No larvae were seen 3 days after infection.

Recovery of worms from small intestines

The small intestines of control and immune mice were examined daily between 1 and 10 days after infection (Fig. 3). In control animals, a few worms were detected in the intestines after 2 days, then the numbers increased rapidly on day 3 and were seen to peak 5 days after infection. Only female adult worms were found. The maximum percentage of adult worms recovered from the intestines at any one time was 21% of the number of larvae applied. The number of adult worms in the small bowel declined after day 5; 10 days after infection, none could be found. Small numbers of eggs which rapidly hatch into rhabditiform larvae were first seen in the intestines of control mice 4 days after infection. Rhabditiform larval numbers reached a peak on day 8 then declined rapidly (Table 1).

TABLE 1—RHABDITIFORM LARVAE RECOVERED FROM THE SMALL INTESTINES OF CONTROL AND IMMUNE MICE

Days after infection	Control mice Mean ± S.E.M.	Immune mice Mean ± S.E.M.
4	880 ± 400	79 ± 29
5	12,700 ± 2900	880 ± 420
6	33,600 ± 16,900	200 ± 51
7	15,800 ± 7700	0 0
8	56,500 ± 21,500	0 0
9	4500 ± 3600	0 0
10	260 ± 260	0 0

In immune mice, worms were first found 3 days after infection, rose slowly to a peak on the fifth day, and were no longer detected on the seventh day after infection. The maximum percentage of adult worms recovered was 3·7%. Worm numbers were significantly lower ($P < 0.005$) than those in control mice on days 3–7. Very few rhabditiform larvae were seen and then could only be found 4, 5 and 6 days after infection (Table 1). These numbers were significantly less ($P < 0.001$) when immune mice were compared with control mice.

Fecundity

Fecundity was estimated by calculating the ratio of the number of rhabditiform larvae to female adult

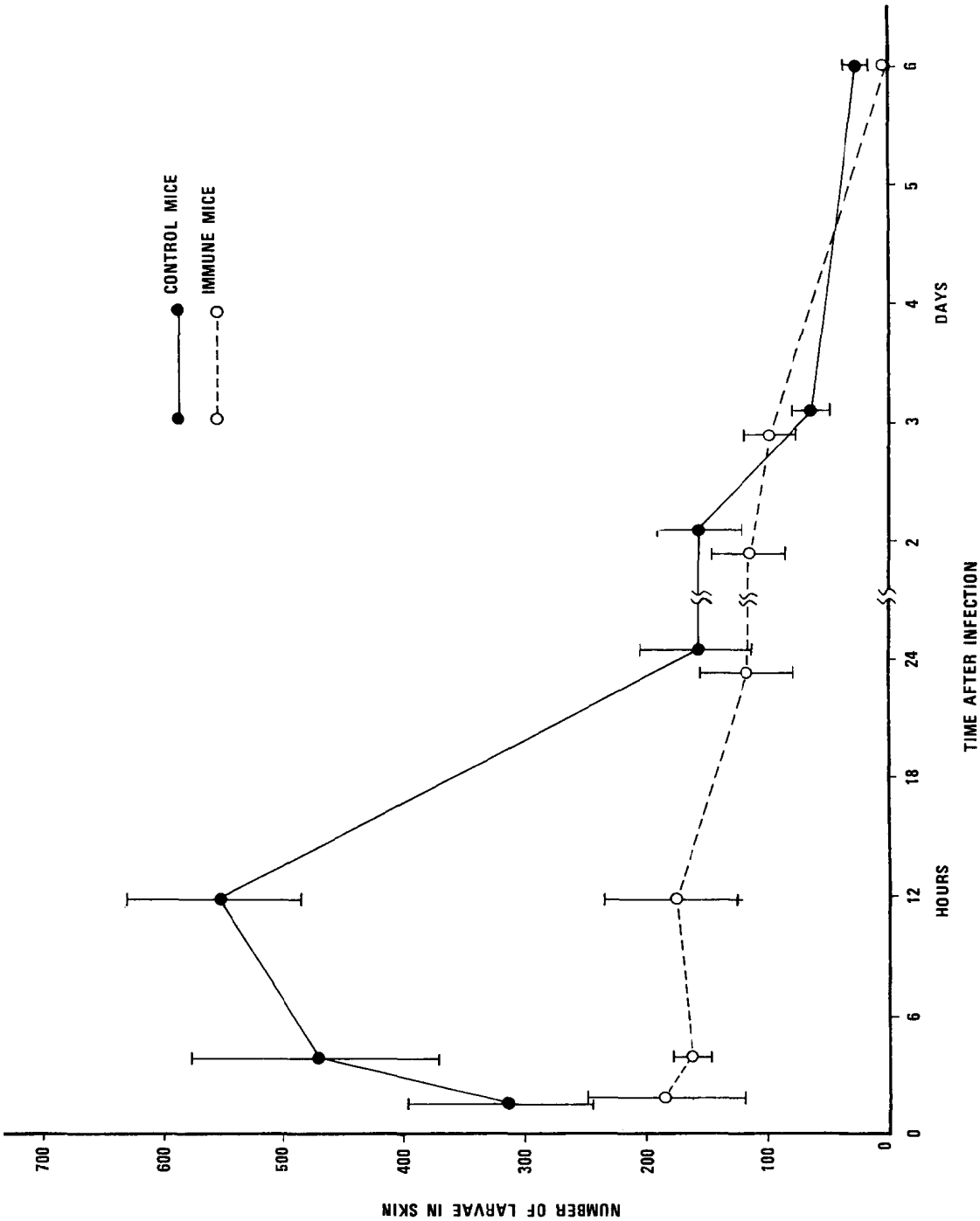

FIG. 1. Larvae recovered from the skins of mice following a primary (●——●) or secondary (○- - -○) infection with filariform *S. ratti* larvae. Values expressed as mean ± S.E.M.

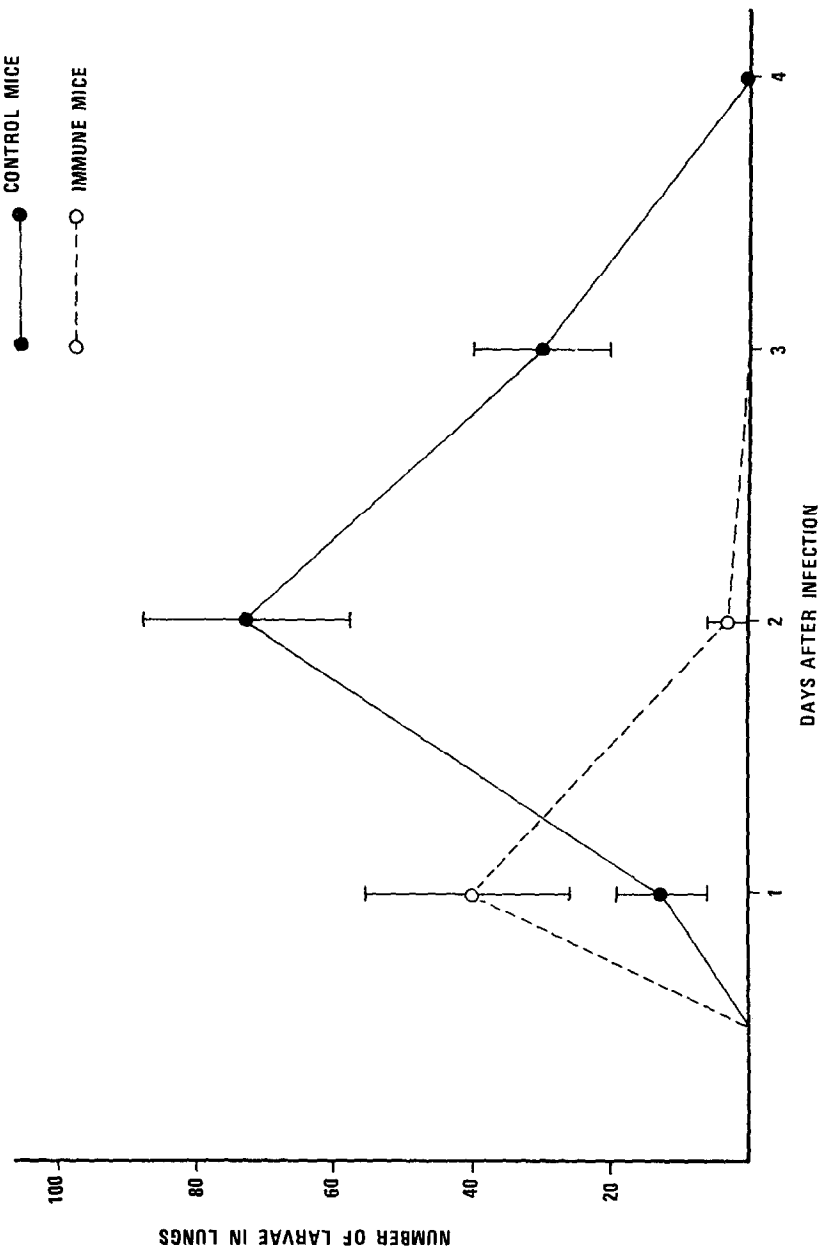

FIG. 2. Larvae recovered from the lungs of mice following a primary (●——●) or a secondary (○- - -○) infection with filariform larvae. Values expressed as mean ± S.E.M.

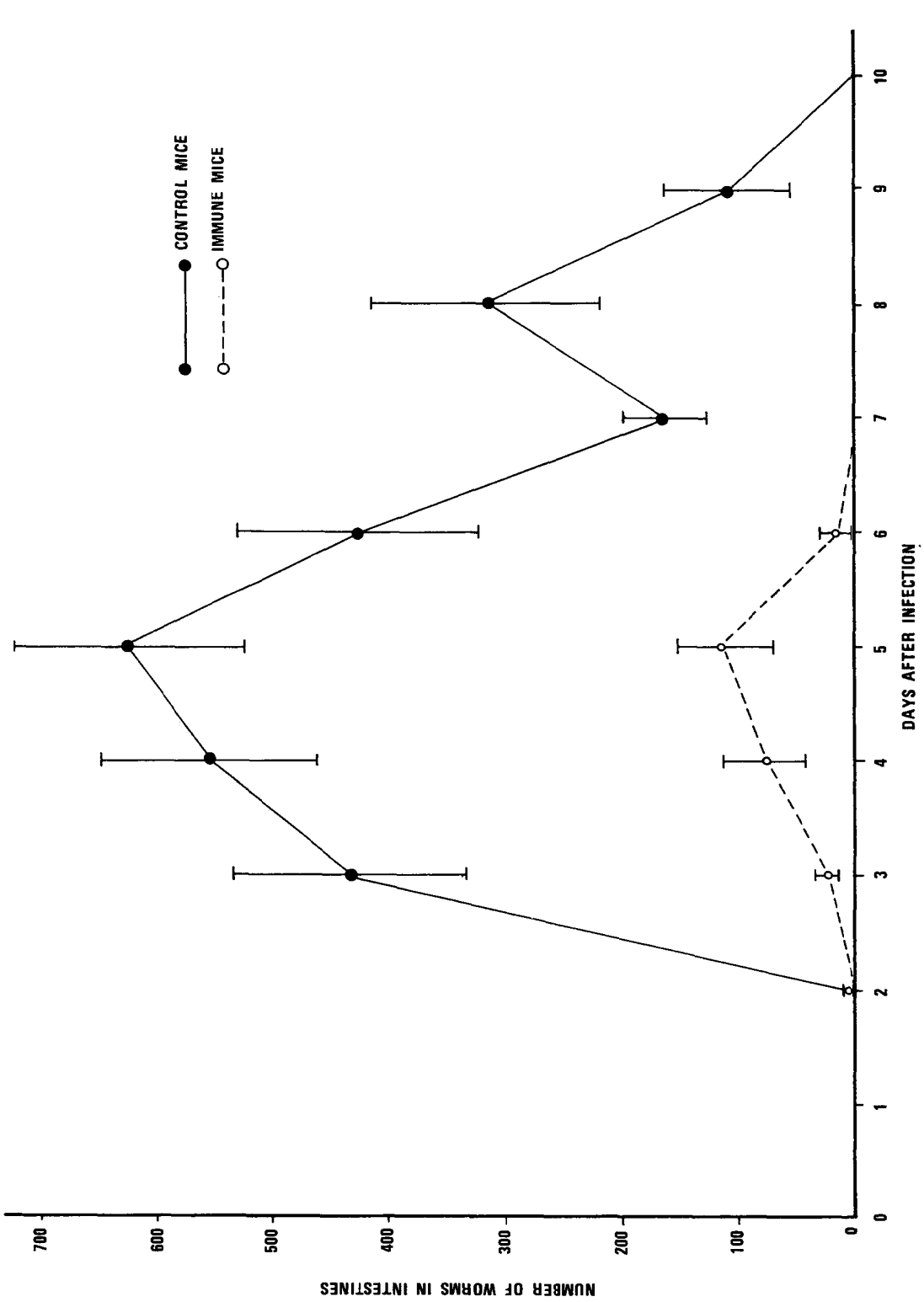

FIG. 3. Adult worms recovered from the small intestines of mice following a primary (●——●) or a secondary (○ - - - ○) infection with filariform larvae. Values expressed as mean ± S.E.M.

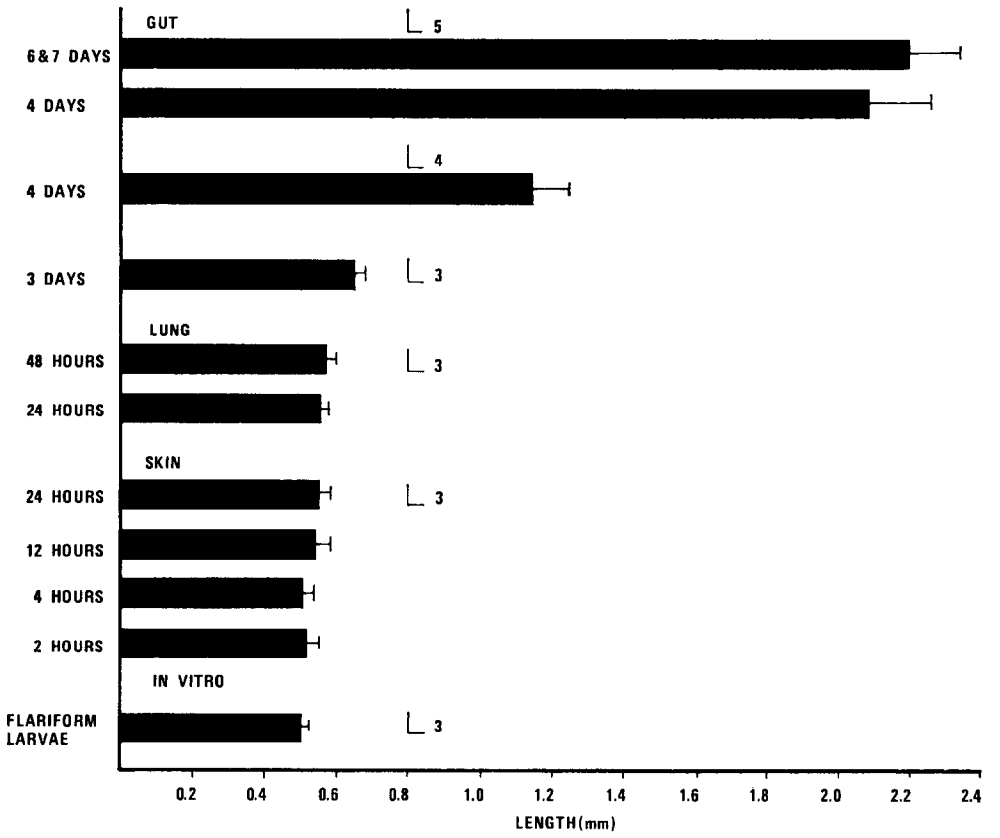

FIG. 4. Length of worms recovered from skin, lungs and small intestines of mice with a primary infection of *S. ratti*. Filariform larvae are designated L_3 and successive moults are indicated by L_4 and L_5. Worm lengths are expressed in millimetres ± S.D.

worms in each intestinal specimen. The fecundity of adult worms recovered from control mice was measured 5 and 6 days after infection, while that of worms from immune mice was measured on day 5 only. The mean number of rhabditiform larvae per adult worm in control mice was 19·6 ± 2·1 (S.E.M.) and 63·1 ± 14·2 on days 5 and 6, respectively. The fecundity of adult worms in immune mice was significantly less ($P < 0·001$), there being 6·7 ± 1·5 (S.E.M.) rhabditiform larvae per adult worm.

Worm lengths

The site and time of moulting was determined by measuring the lengths of worms recovered from the skin, lungs and intestines of control mice (Fig. 4). Filariform larvae prior to infection were 0·50 ± 0·02 mm (mean ± S.D.). Two hours after infection, larvae measured 0·52 ± 0·03 mm. Worm length was observed to increase slowly over the next 3 days in larvae recovered from the skin, lungs and gut. On day 3, larvae recovered from the intestines measured 0·65 ± 0·03 mm. A moult then occurred in the small intestine between the third and fourth days. On day 4, two populations of worms were found, their mean lengths ± S.D. being 1·20 ± 0·10 mm and 2·10 ± 0·17 mm, thus indicating that the final moult began on that day.

DISCUSSION

This murine model of strongyloidiasis has allowed quantitation of the numbers of larvae in the skin, lungs and small intestines at various times after a primary infection with *S. ratti*. These findings were then compared with those in animals which were re-infected four weeks after the original exposure.

In mice infected for the first time, larvae appeared to penetrate the skin fairly rapidly. Approximately three-quarters of the larvae had migrated away from the skin by 24 h after infection. Thereafter, larval numbers declined slowly with increasing duration of infection. In contrast to these observations, Abadie (1963) and Wertheim & Lengy (1965) could not find larvae in the skin beyond three days and 24 h,

respectively, after infection. This may indicate that their findings reflect either a sampling error because of an insensitive, non-quantitative technique, or that the migratory behaviour of *S. ratti* larvae in rats is faster.

It has been assumed by most workers that after penetration of the skin, larvae enter the blood vessels and pass directly to the lungs. Recently, however, Tada and co-workers (1979), using a different strain of *S. ratti*, have provided some evidence that there is a more complex route. They have suggested that larvae pass from the site of inoculation through the subcutaneous tissues to the nasomaxillary and intracranial regions of the head, thence to the small intestine, presumably via the blood vessels and lungs. We have not investigated this possibility in the present study.

We first found larvae in the lungs 24 h after infection; this compares with times of 12 and 16 h noted in rats by Abadie (1963) and Wertheim & Lengy (1965), respectively. The maximum number of worms found in the lungs was only 2·4% of those applied. Since the percentage of worms ultimately recovered from the gut was much greater than this, and as the peak appearance of worms in the lungs at 48 h was also the time when worms first appeared in the gut, it appears likely that larvae spend only a short period, perhaps a few hours in the lungs.

Worms began appearing in the small intestines 48 h after infection. This timing is similar to that noted in rats; Abadie (1963) first found worms in the gut 48 h after infection while Wertheim & Lengy (1965) observed them after 34 h. The maximum number of worms recovered from the intestines was 21%. This is similar to the 24% recovery after transmucosal infection and 27% recovery after subcutaneous injection noted in rats by Sheldon (1937a), but is considerably less than the 40–60% recovery achieved after subcutaneous injection by Moqbel & Denham (1977). Adult worm numbers began to fall six days after infection, and by 10 days, no adult worms could be found. This expulsion of adult worms in mice is much quicker than is seen in rats (Sheldon, 1937b; Moqbel & Denham, 1977) and confirms our previous observations on the excretion of larvae in mouse faeces (Dawkins *et al.*, 1980).

The number of moults and sites of moulting of *Strongyloides* larvae in the host are controversial. Lucker (1934) observed only one parasitic moult in *S. ransomi* whereas Basir (1950) observed two parasitic moults in *S. papillosus*. Abadie (1963) makes no direct mention of moulting of *S. ratti* but Wertheim & Lengy (1965) described two parasitic moults. Faust (1933), Basir (1950), Reesal (1951) and Turner, Shalkop & Wilson (1960) observed ecdysis of *Strongyloides* larvae in the lungs of the host. In contrast, Wertheim & Lengy (1965) considered that the vast majority of larvae moulted in the small intestine. The sizes of the different stages of *S. ratti* are distinctive, and we have clearly shown in the present study that two moults occur and both of these take place in the small intestine three and four days after infection. The appearance of a few rhabditiform larvae on the fourth day after infection which is also the day on which adult worms were first seen, suggests that the production and release of rhabditiform larvae begins within a number of hours of maturation.

When the migration of larvae in previously-exposed mice was investigated, quite different observations were made. The maximum number of worms recovered from the skins of these animals was much reduced, being one third to one quarter of the numbers seen in normal mice. Since the first counts were made two hours after exposure, this indicates either that less worms were able to penetrate the skins of these mice, or that worms which penetrate leave more quickly. Lewert & Lee (1954) have shown in histological studies that there is a marked immediate hypersensitivity reaction in the skin of immune animals. They suggested that this response may inhibit penetration of larvae. They did not provide any evidence for this contention, however, and indeed, it is hard to conceive how such a reaction might inhibit entry. Alternatively, it could be considered that the oedema and increased vascularity of the immediate hypersensitivity reaction would facilitate the rapid removal of larvae from the site of penetration in the skin. In an attempt to differentiate between these two views, we infected normal and previously exposed animals, then one hour later, counted the numbers of worms left on the skin surface and recovered after vigorous rubbing and washing. Very few worms were seen in either group, thus suggesting that the latter hypothesis may be more likely.

Further support for this concept is provided when the kinetics of larvae in the lungs are considered. There was a suggestion that larvae may reach the lungs earlier in immune animals for, although not statistically significant, greater numbers of larvae were seen at 24 h. There was, however, a subsequent marked reduction in recovery of worms from these mice 48 and 72 h after infection as compared with control animals. Moqbel & Denham (1977) have suggested that larvae are killed in the lungs of immune rats, although they were not able to provide evidence for this thesis. The lungs provide, theoretically, a convenient place for phagocytic and other cells to be marshalled, attack and destroy migratory larvae. Whether or not larvae recovered from the lungs of immune mice are less than or greater than the numbers seen in primary infections, presumably depends upon the degree of obstruction to passage and the rate of destruction of larvae. Thus, our failure to find many larvae in the lungs of immune mice 48 and 72 h after infection suggests rapid destruction of worms at this site.

Worm numbers in the small intestines of immune

mice were much reduced, there being less than one fifth of the number of worms that were seen in normal mice. This indicates either that larvae failed to reach the small intestine, perhaps as a result of destruction of larvae in the lungs, or that many of the larvae which reached the gut failed to establish themselves and were passed in the faeces. Not only did less worms establish themselves in the small intestines, but those worms which did mature were expelled more rapidly than were adult worms in normal mice. Furthermore, those worms which did reach maturity had a greatly impaired fecundity. A similar phenomenon has been observed in immune animals infected with *Trichinella spiralis* (Despommier, Campbell & Blair, 1977). Thus, the excretion of larvae in the faeces of immune animals is greatly reduced as a consequence of both reduced adult numbers and decreased fecundity.

In conclusion, it seems likely that the immune host influences the behaviour of *S. ratti* in a number of ways. We have speculated that the departure of larvae from the skin is accelerated as a result of an immediate hypersensitivity inflammatory reaction, and that there may be destruction of a proportion of larvae in the lungs. There is no doubt that less worms reach maturity in the gut, and of those which do, their fecundity is impaired and they are expelled more quickly. Our speculations need to be further investigated and confirmed or negated, and the mechanisms by which these various defensive processes are bought into operation remain to be elucidated.

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