MIGRATIONS OF NEMATOSPIROIDES DUBIUS (NEMATODA) IN THE MOUSE

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ABSTRACT

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The migratory abilities of the infective larvae of Nematospiroides dubius were studied by introducing the larvae into the mouse host at the following sites; the surface of the skin, the systemic venous circulation and the peritoneal cavity. Oral infections were also studied to determine if larvae migrate from the intestine. Larvae were recovered from the organs and tissues by pepsin digestion.

Only a few of the infective larvae were able to penetrate the skin and complete the migration from the subcutaneous layers of the skin to the intestine. When infective larvae of N. dubius were injected into the subcutaneous layers of the skin most of the larvae did not enter the vascular system and only a few were found in the small intestine, but the larvae did undergo random migration in the tissues. Larvae injected into the tail vein were trapped in the lungs and migrated from the lungs to the digestive tract. Larvae of N. dubius possessed the same ability as the skin penetrating larvae of Nippostrongylus braziliensis to migrate from the lungs to the digestive tract but fail to enter the vascular system from the tissues.

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By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Nematospiroides dubius is an intestinal nematode of mice which has come to the attention of several investigators in recent years because of the pathology and immunological response caused by this worm. Lepak (1962) suggested that some of the infective larvae of N. dubius introduced into the subcutaneous layers of mouse skin were able to migrate to the intestine and mature into adult worms. This indicated that N. dubius may have some of the tissue penetrating abilities of the skin penetrating nematode Nippostrongylus braziliensis.

The purpose of this investigation was to determine what abilities for tissue penetration and migration N. dubius shares with N. braziliensis. To do this infective larvae of N. dubius were injected into various tissue sites and the locations of the larvae followed to determine the extent and route of their migration.

LITERATURE REVIEW

Nematospiroides dubius was originally described by Baylis (1926) from the small intestine of the wood mouse (*Apodemus sylvaticus*) and placed in the family Heligmosomatidae. *N. dubius* has since been reported from other wild mice like *Peromyscus maniculata* (Ehrenford, 1954) and *Mus musculus* (Spurlock, 1943) and can be easily maintained in several strains of laboratory mice.

The life cycle of *N. dubius* as described by Spurlock (1943), Ehrenford (1954), Baker (1954), and Callizo (1962) takes about 15 days under optimal conditions. Fahmy (1956) investigated the free-living stages of *N. dubius* and found that the eggs hatch within 24 hours after being passed with the feces. Third stage larvae were found 48 to 56 hours later, but they were not infective until at least 5 days after the eggs hatched. Fahmy stated that mice became infected by ingesting the sheathed third stage larvae. Liu (1965a) found larvae in the mucosa and submucosa of the stomach until 36 hours after ingestion. Exsheathed larvae were found by Baker (1954) and Liu (1965a) in the crypts of Lieberkühn of the small intestine 24 hours after ingestion. The larvae first penetrated into the mucosa through the crypts causing necrosis at the sites of entry. This was followed by migration into the muscularis where, according to Ehrenford (1954), they came to rest against the longitudinal muscle

layer. Baker (1954) believed that the transverse orientation of the larvae to the longitudinal axis of the small intestine indicated that the larvae encyst in the circular muscle layer. Liu (1965a) reported that by the second day of infection petechial and ecchymotic hemorrhages were seen in the muscularis and worms were found at the sites of hemorrhage. Baker (1954) stated that the size and number of sites of hemorrhage increased up to the third day of infection because of the rapid growth of the larvae in the muscularis and the entry of more larvae into the tissues. Leucocytic infiltration began causing the sites of hemorrhage to become opaque. Progressive tissue degeneration and leucocytic infiltration was followed by encapsulation of the larvae in the muscularis during the second and third day of infection. The sites of encapsulation increased in size until the twelfth day due to the continued infiltration of leucocytes. During the period between 3 to 12 days after infection, the gut wall was greatly distended. Spurlock (1943) reported that some animals died because of perforation of the gut wall. Deaths also occurred from mass hemorrhage of the mucosa and fecal occlusion of the intestine of heavily infected mice. The mass hemorrhage of the mucosa occurred when the larvae returned to the lumen of the small intestine, 6 to 8 days after infection. The adults attached to the mucosa with the greatest number near the pyloric valve. Fifteen to 16 days after infection unembryonated eggs were

passed in the feces of the mice.

Lesions developed in other organs besides the intestine 3 to 9 days after infection. Spurlock (1943) reported that the liver, kidneys, and spleen were pale in color and that the spleen was enlarged. There was "a slight serous to serosanguinous exudate in the body cavity". Baker (1963) and Liu (1965) both reported that leucocytosis and spleenomegaly occurred early in the infection due to "necrotic substances" produced by the larvae in the intestinal wall. Liu reported that the mesenteric lymph glands were heavily infiltrated and hyperplastic. The endothelial cells in the branches of the portal viens were swollen and neutrophils were evident around these vessels. Other neutrophilic foci, which were observed in the parenchyma of the liver, increased in numbers until the ninth day. Twelve to 21 days after infection these lesions became progressively fibrotic; first those in the capsule of the liver followed by those near the branches of the portal vien sinusoids. Liu failed to find larvae in sections of the mesenteric lymph glands, liver, or spleen showing pathology.

Only a few experiments to determine the migration pattern of the infective larvae introduced at abdominal sites in the host have been published. Roman (1951) examined histological sections of skin of rats exposed to larvae. Since he did not find larvae he concluded the larvae would not penetrate the skin. Baker (1955) injected third stage larvae both into subcutaneous areas of the abdomen skin and

into the peritoneal cavity. Some of the larvae invaded and encysted in the mesenteries of the mice and showed signs of growth, but no evidence of sexual development was observed. He also noted subcapsular lesions lacking larvae in the liver and spleen and stated they were due to larval penetration of the capsule. Lepak et. al. (1962) made incisions in the flanks of mice which exposed the subcutaneous areas of the skin. Larvae were introduced into these incisions and the skin was closed with sutures. When some of the mice were autopsied 14 and 28 days later, worms were found in the subcutaneous tissues and the gut. Stunted worms were found in the subcutaneous tissues 185 days after initial infection. The males had well developed bursae and some of the females contained infertile eggs. Lepak (1962) wrote, "Further experiments will be necessary to show whether the few worms found in the gut were the result of migration" from the skin.

The life cycles of only a few members of the Family Heligmosomatidae are known, but two routes of larval entry into the host have been demonstrated: skin penetration and ingestion (Skrjabin et. al. 1952). Yokagawa (1922) and Schwartz and Alicata (1943) reported the Nippostrongylus braziliensis, which normally infects its host by skin penetration, can infect rats by the oral route. Longistriata musculi which normally enters the mouse by ingestion has also been reported by Schwartz and Alicata (1935) to infect by skin penetration. Unfortunately their observation have not been repeated by other investigators. The recovery of

N. dubius adults from the small intestine after the larvae were placed in the subcutaneous tissues indicates that both routes of infection might be possible for this species (Lepak, 1962).

For infection by either the oral or cutaneous route a nematode must have certain adaptations or abilities; such as the ability to select the successful route of migration and to penetrate tissues. To be a successful skin penetrator like N. braziliensis the infective larvae must be able to exsheath in the external environment or in the absense of stimulatory conditions peculiar to the digestive tract and penetrate the host's skin. Enzymatic action may be required for penetration as has been demonstrated with Strongyloides, but the infective larvae of N. braziliensis apparently lack most common enzymes associated with penetration (Lewert 1954). Once the larvae has entered the host it must be able to follow a migratory path to the site of final maturation, which is the lumen of the gut in most cases. The larvae of N. braziliensis, like most skin penetrating forms, must enter the vascular system to be carried to the lungs. They must be retained in the capillary bed of the lungs or else they would be carried to all parts of the body by the systemic circulation. Retention in the lungs may be due to their inability to pass through the capillaries. The larvae in the lungs gain access to the air passages, either by active migration or by rupture of blocked capillaries,

where they migrate or are carried by ciliary action up the trachea and are eventually swallowed. Once in the gut the larvae actively enter or adhere to the mucosa of the small intestine.

There would seem to be less adaptation necessary for oral infection. Forms ingested orally like N. dubius are stimulated to exsheath by the conditions of the physio-chemical environment encountered in the host. Extensive tissue migrations are not common after oral infections. Some species in the Trichostrongyloidea like N. dubius may penetrate into the mucosa and enter the muscle layers and eventually return to the lumen.

METHODS AND MATERIALS

The parental stock of Nematospiroides dubius was obtained from the Ely Lilly Company and has been maintained in this laboratory for about 3 years. All mice used for experimental purposes were female albino mice of the Swiss Webster strain weighing between 15 and 20g. Larvae were obtained by smearing moist fecal pellets containing eggs on strips of filter paper and culturing them in centrifuge tubes as described by Sadig (1964). The infective, 3rd stage larvae were collected from 6 day old cultures, suspended in 0.85% NaCl and washed free of most of the fecal debris by at least 4 low speed centrifugations and decantations. After the final wash the larvae were suspended in 3 to 7 ml of 0.85% NaCl and stored at 4°C in 50 ml Erlenmeyer flasks stoppered with cotton plugs.

Stock infections and some experimental infections were given by oral intubation of infective larvae using a two inch, blunt, curved 18 gauge needle attached to a 1 ml glass tuberculin syringe. For stock infections mice were given 200 to 300 larvae each and the infection was transferred at 3 month intervals.

Except for certain oral infections or when otherwise specified, all larvae used for experimental infections were exsheathed. Nearly one hundred percent exsheathment of the larvae was obtained by first suspending the larvae in a 0.154 M KCl solution adjusted to a pH of 1.7 with 0.154 M HCl.

One ml of the larval suspension was then placed in a 10 x 13 mm test tube. Rubber serum bottle stoppers were used to seal the tubes. Two hypodermic needles were inserted into the tube, one as an inlet and the second as an outlet for gas. A mixture of 5% CO_2 and 95% N_2 was bubbled into the larval suspension for ten minutes before the needles were removed. The larvae were then incubated at 39°C for 40 minutes while exsheathment took place. After exsheathment the larvae were washed 4 times by a series of low speed centrifugations and decantations. The best exsheathment occurred after the larvae had been stored for at least 7 days at 4°C.

The larval concentration in the suspensions used for infection of mice was determined by withdrawing at least four 0.05 ml or 0.1 ml samples with a 1 ml tuberculin syringe, counting the larvae in a Scott Chamber, and averaging the counts. The larval suspension was adjusted to give the desired number of larvae in 1 ml of suspension.

Experimental oral infections were given to the mice in the same manner used in stock infections. The abdomen of each mouse was rubbed thoroughly with 70% ethyl alcohol before and after each subcutaneous and intraperitoneal injection of larvae to kill any larvae that might have been accidentally deposited on the skin. For intravenous injections mice were restrained in a box with a U-shaped notch to allow the tail to protrude. The injections were given in the right lateral tail vein using a 26 guage needle. The tail was wiped before and after each injection with 70% ethyl alcohol. To test the

ability of the larvae to penetrate the skin, the mice were anesthetized with 0.1 cc of a 1:100 dilution of Halatal (1 grain Na pentobarbital/cc carrier) by intraperitoneal injections. The abdomen of each mouse was shaved with electric clippers fitted with a size 40 head. Animals which received cuts or abrasion from shaving were not used. After shaving the mice were tied to a table with thread and tape and the exposed abdomen was wiped with 70% ethyl alcohol. When the alcohol was dry the larvae were spread over the shaved area. The mice were exposed to the exsheathed infective larvae for 45 to 60 minutes before the abdomen and the surrounding fur was thoroughly wetted with 70% alcohol. After 5 minutes exposure to the alcohol the mice were placed back in their cages. The mice were kept at 39° from the time they were anesthetized until they were totally revived to prevent a drop in body temperature.

To determine the fate of the larvae introduced at the various sites the lungs, trachea, esophagus, stomach, small intestine, liver, and kidneys were checked for larvae. The skin at the abdominal site of infection was checked in those experiments involving skin penetration, subcutaneous injections and peritoneal injections. The subcutaneous tissues and the abdominal muscle layers were included in each of the skin samples examined.

The larvae were recovered after pepsin digestion of the tissues. The organs and tissues were placed in separate 114 x 25 mm (40 ml) glass screw cap vials and chopped into

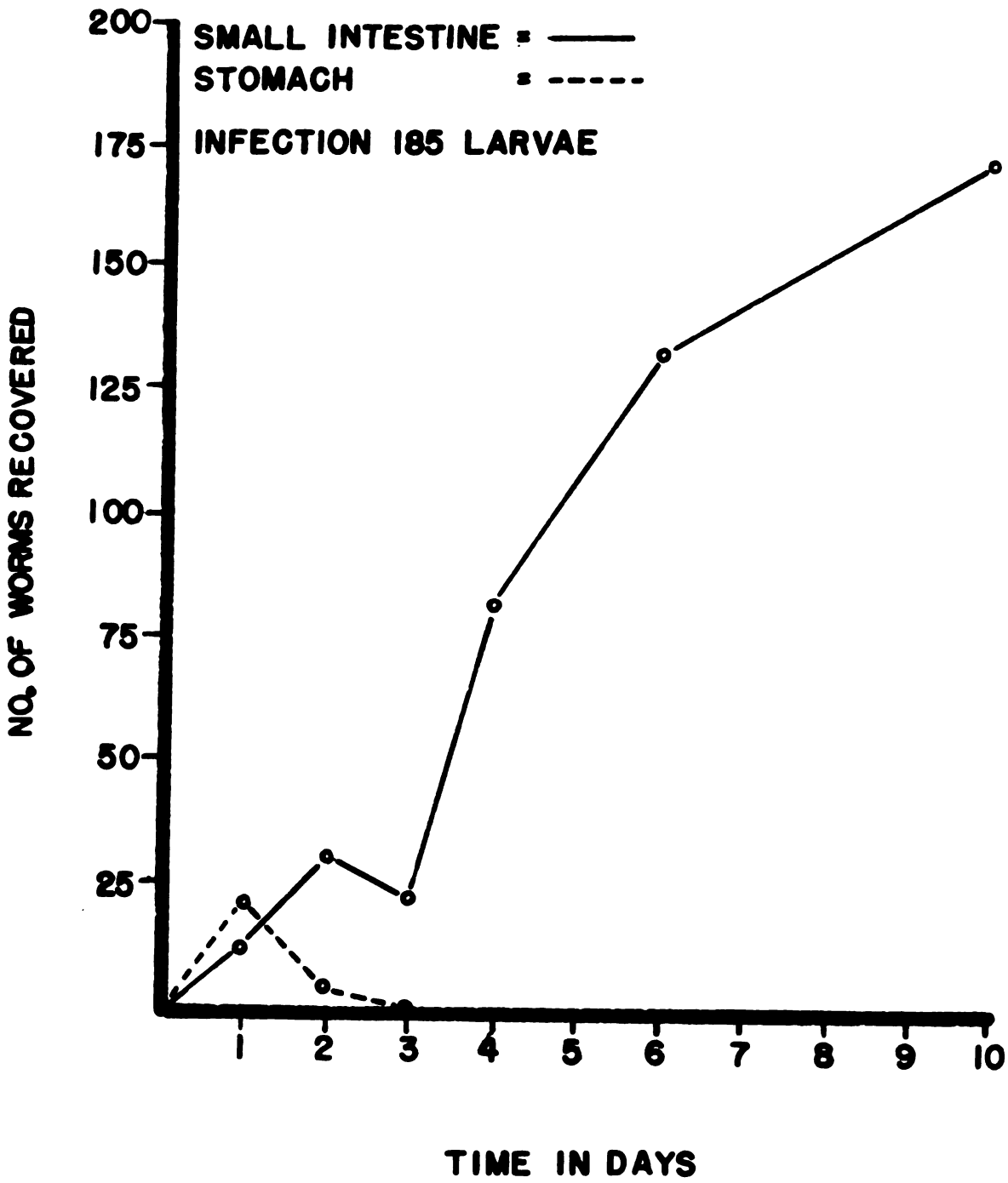
small pieces with a pair of scissors. A pepsin solution (1% pepsin, 0.5% HCl, and 0.85% NaCl) was added until the fluid filled all but the upper 1 cm of the vial. The vials were capped and agitated in a shaker box at 39°C in a room incubator for 6 hours. After digestion the contents of the vials were placed in 50 ml centrifuge tubes and centrifuged at 72 X G for 5 minutes. The supernatant was carefully decanted and the settled contents were resuspended with 0.85% NaCl and centrifuged again. This was repeated until the supernatant was relatively clear. All except the bottom 15-20 ml of supernatant was then decanted from the centrifuge tube. The remaining volume was stirred and poured into a petri dish marked off in ½ inch squares. In the experiments that large numbers of larvae were recovered only 20% of the dish was examined. When larvae were few, the entire dish was examined. All counts were made using an A.O. Cycloptic dissecting microscope.

RESULTS

I. Oral Infection

Mice were infected orally with sheathed infective larvae of N. dubius in order to confirm prior descriptions of larval migration in the host, to determine the efficiency of the techniques employed in recovering worms, and to search for larvae in other body organs that might indicate aberrant migrations. Eighteen mice were infected orally with 185 larvae each and autopsied in groups of 3 at 1, 2, 3, 4, 6, and 10 days after infection. During the first 3 days less than 20% of the larvae were recovered from the stomach and small intestine (Fig. 1). Digests of the stomachs revealed the greatest number of larvae at 24 hours. Only a few larvae were found in this organ on the second and third days after infection. Larvae were in the intestine as early as 24 hours after infection, but the recoveries of larvae were low and variable during the first 3 days of the infection. After 3 days the percent recovered increased with the age of the infection. Ten days after infection 94% of the larvae were recovered as adults from the small intestine. The lungs, trachea, esophagus, liver and kidneys of all animals employed in this experiment were removed, digested, and examined to determine if larvae migrated from the small intestine. All examinations for larvae were negative. The lungs, liver, and kidneys were selected because of

Figure 1. Location and numbers of worms recovered at intervals of time after oral intubation of exsheathed infective larvae of *N. dubius*. Each point represents an average from 3 mice.


SMALL INTESTINE = ——
STOMACH = - - - - -
INFECTION 185 LARVAE
NO. OF WORMS RECOVERED
200
175
150
125
100
75
50
25
1
2
3
4
5
6
7
8
9
10
TIME IN DAYS

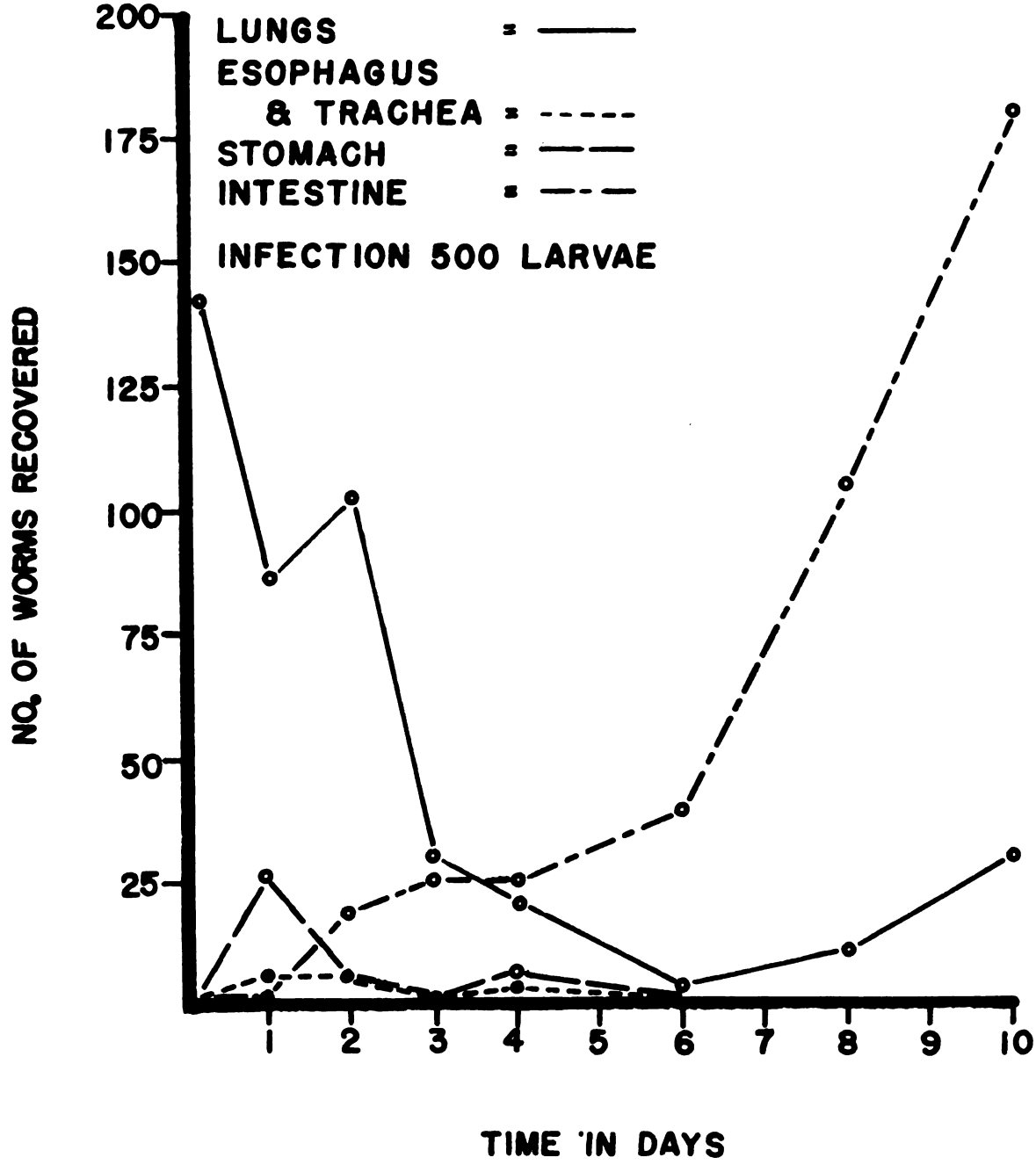
their large blood flow and extensive capillary beds which would be expected to filter out larvae entering the vascular system.

It was apparent from the results of this experiment that many of the young larvae were lost due to their small size or to the action of the pepsin solution on the larvae.

II. Intravenous Injection

Exsheathed infective larvae of N. dubius were injected intravenously into mice to determine if they were capable of a lung to digestive tract migration. Large numbers of larvae were found in the lungs 3 hours after intervenous injection (Fig. 2) and the number of larvae remained high for 2 days. From 3 days on the lung recoveries decreased, but some larvae persisted and a few even showed development into late fourth or early fifth stage larvae. Larvae were first recovereed from the trachea, esophagus, and stomach 24 hours after infection. Small numbers of larvae were found in these organs up to 6 days after infection. There was no marked increase in the number of larvae in these sites 2 to 3 days after infection when the majority of larvae disappeared from the lungs. The worms were first seen in the small intestine 2 days after infection. After 2 days the number of worms recovered from the small intestine rose gradually at first with a dramatic increase in recoveries after 6 days, the time larvae migrate out

Figure 2. Location and numbers of worms recovered at intervals of time after intravenous injection of infective larvae of N. dubius. Each point represents an average of 3 mice.


LUNGS =
ESOPHAGUS & TRACHEA =
STOMACH =
INTESTINE =
INFECTION 500 LARVAE
NO. OF WORMS RECOVERED
200
175
150
125
100
75
50
25
1
2
3
4
5
6
7
8
9
10
TIME IN DAYS

of the intestinal wall into the lumen. Checks of the liver and kidneys failed to find any larvae, indicating the larvae had not entered the systemic circulation. Thirty-three percent of the larvae were found as adults 10 days after infection. The larval recoveries were generally lower than those of the adults, with the lowest number of larvae recovered between 3 and 6 days after infection. The results show that at least some of the exsheathed larvae are capable of a lung to digestive tract migration and that most of the larvae given intravenously have a period of residency in the lungs.

III. Intraperitoneal Injections

Larvae were injected into the peritoneal cavities of mice to determine: (1) if they could migrate to the small intestine to mature to adults and (2) if they reached the intestine, what migratory route they would follow to the intestinal wall. A group of 24 mice were injected with 320 larvae each and sacrificed in groups of 3 at predetermined times. Twenty-seven larvae were recovered in peritoneal washings 3 hours after infections, but no larvae were recovered from peritoneal washings of mice killed at 6 hours or 1, 2, 3, 6, 10, or 18 days after intraperitoneal injections. Examinations of the abdominal tissues, lungs, trachea, esophagus, small intestine, liver and kidneys after pepsin digestion failed to demonstrate any larvae. Digestions of the stomachs led to the recovery of 9 undeveloped

larvae from the mice autopsied at 3 days and 3 undeveloped larvae from those examined 6 days after infection.

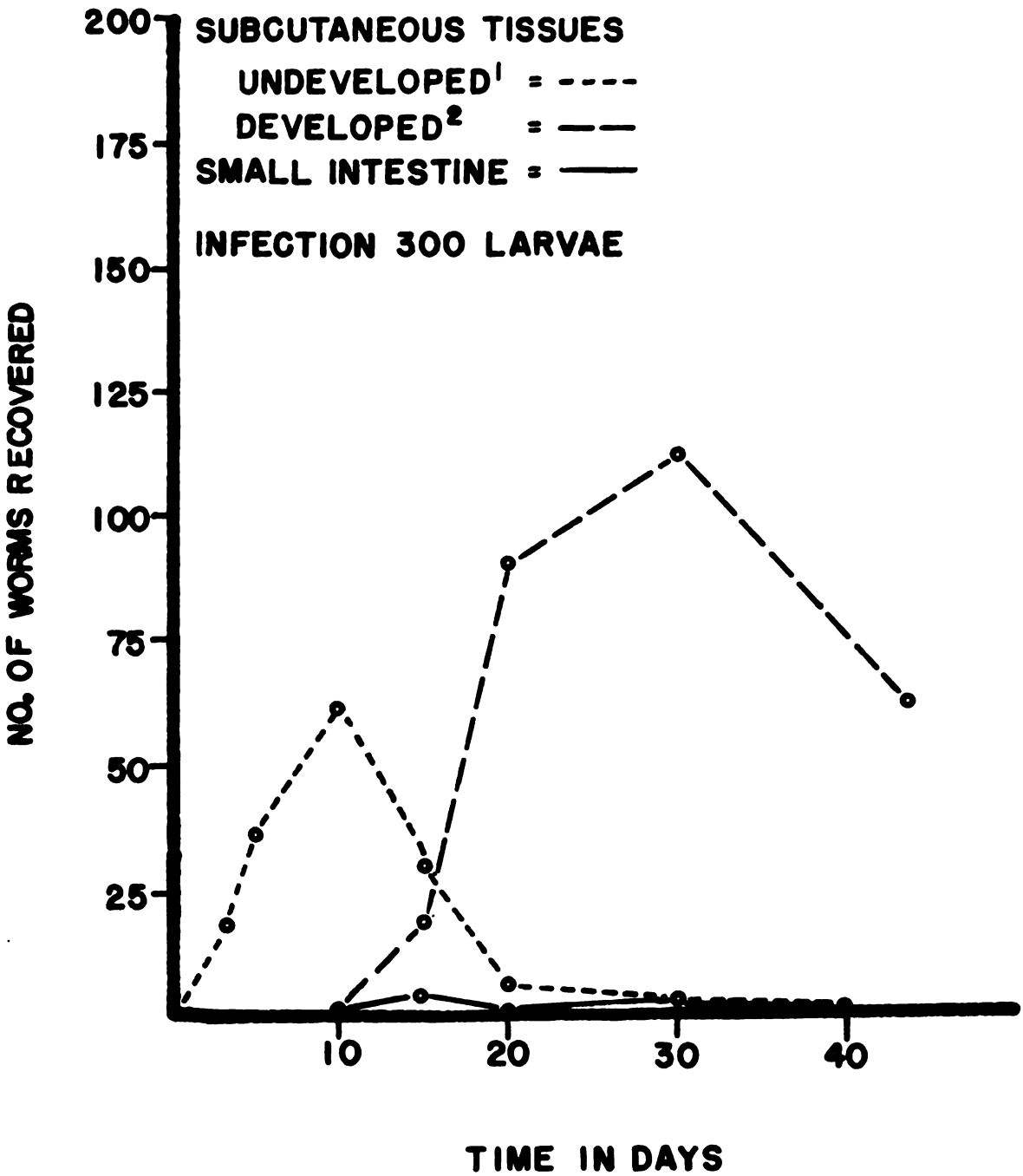
IV. Subcutaneous Injection

The ability of larvae to migrate from the subcutaneous tissues was checked by injecting larvae under the abdominal skin and attempting to find them in pepsin digests of the abdominal skin and muscle, lungs, trachea, esophagus, stomach, small intestine, liver, and kidneys. A group of 21 mice were given subcutaneous injections of 300 exsheathed larvae. They were sacrificed in groups of 3 at 3, 5, 10, 15, 20, 30, and 45 days after infection. Most of the recovered larvae were found in the subcutaneous and muscle layers of the abdomen, however, lateral migration did occur. During the ten day period after infection mostly undeveloped larvae (third stage to early fourth stage larvae) were recovered from the subcutaneous tissues (Fig. 3). After this period the number of undeveloped larvae decreased while the number of developing larvae (late fourth stage to fifth stage) increased. Many of the later worms were fully mature as was demonstrated by the well developed bursae of the males and the presence of eggs in the females and surrounding tissues. Few larvae or adults were recovered from the small intestine and no recoveries were made from digests of the lungs, trachea, esophagus, stomach, liver, or kidneys. In one mouse 2 larvae were found in the mesenteries between the pancreas and the small

Figure 3. Location and numbers of worms recovered at intervals of time after subcutaneous injection of exsheathed infective larvae. Each point represents an average from 3 mice.

[1]Third to early fourth stage larvae.

[2]Late fourth stage larvae to fifth stage worms.


SUBCUTANEOUS TISSUES
UNDEVELOPED[1] = ----
DEVELOPED[2] = — —
SMALL INTESTINE = ——
INFECTION 300 LARVAE
NO. OF WORMS RECOVERED
200
175
150
125
100
75
50
25
10
20
30
40
TIME IN DAYS

intestine. The highest recovery of adults from the small intestine was 1% of the larvae injected. Forty-five percent of these same larvae were recovered as adults from 5 mice after oral infection. The route of migration that was followed to reach the small intestine after subcutaneous injection of larvae could not be determined because of the small number of successful migrants.

V. Skin Penetration

Exsheathed larvae of N. dubius were placed on the shaved abdomens of mice to determine if they could penetrate the skin. In one experiment 1000 larvae were placed on the exposed abdomens of 8 mice. Three of these mice were autopsied after 10 days and the abdominal tissues, lungs trachea, esophagus, stomach, small intestine, liver, and kidneys were digested with pepsin solution. Eighty-four (3%) of the larvae exposed to the skin were recovered as adults in the small intestine and 6 larvae were found in the abdominal subcutaneous tissues. Eggs were found in the feces of the remaining mice. In an attempt to determine the route of migration to the intestine, the abdomens of 24 mice were exposed to 500 exsheathed larvae each and then autopsied in groups of 3 mice each, 6, 9, 13, 15, 17, 19, and 26 days later. Adult worms were seen in the small intestine at 13, 15, 19, and 26 days, but the adults present accounted for

less than 1% of the larvae exposed to the skin. No worms were found in pepsin digests of the abdominal tissues, lungs, trachea, esophagus, stomach, liver, or kidneys.

VI. Comparison of All Routes

An attempt was made to determine if the variation in recoveries after different routes of infection was not due, in part, to differences between larval cultures. Seven groups of 5 mice each were infected with one of two larval preparations. All of the mice were autopsied 14 days after infection and the number of worms in the small intestine counted. The route of infection, larval culture employed, the presence or absense of sheaths, and the adults recovered in each group are listed in Table 1. The percentage of adults recovered in the groups receiving oral injections was similar. Two groups received intravenous injections; one with exsheathed larvae and a second with sheathed larvae. The percentage of larvae maturing to adults after intravenous injection was much lower than by the oral route, but the recoveries were comparable for infections with sheathed or exsheathed larvae. Mice exposed to infection by skin penetration or by subcutaneous injection both showed very few adult worms, but the percent recoveries were of comparable magnitude (2% and 3%).

The final recoveries of adults in this experiment were comparable to the results of earlier experiments.

It would seem that the variance in infectivity between larval cultures was small.

TABLE 1. Recovery of adult N. dubius from the small intestine after infection by various routes. The percentages are averages of groups of five mice each.

Route of Infection	Larval Culture	Infective Larvae	% Adults Recovered
Per OS	I	230 exsheathed	99%
Intravenous	I	230 exsheathed	41%
Subcutaneous	I	230 exsheathed	3%
Percutaneous	I	230 exsheathed	2%
Per OS	I	230 sheathed	96%
Intravenous	II	220 sheathed	39%
Per OS	II	220 sheathed	94%

DISCUSSION

As a method for recovering Nematospiroides dubius larvae from host tissues, the technique of pepsin digestion had some drawbacks. Only 10-25% of the third and fourth stage larvae could be found, with the lowest recoveries occurring 3 days after oral and subcutaneous infection and 3-6 days after intravenous infection. Increased recoveries were associated with the appearance of late fourth stage larvae. As the larvae matured to adults the recoveries increased after both oral and intravenous infection, exceeding 90% with oral injections. This indicated that the larvae were present but only a small percent were found. Third and early fourth stage larvae could have been lost either in the process of centrifugation and washing of digests or of digestion of the tissues. Partial digestion could alter the specific gravity and contribute to a loss in the centrifugation process if the early stages were more subject to digestion than late stage larvae. When infective larvae of N. dubius were intubated orally the larvae followed the general course of migration described by Spurlock (1943), Ehrenford (1954), Baker (1954), and Callizo (1962). The percentage of larvae that survived to form adults was high, above 90%, whether sheathed or exsheathed larvae were employed. This is comparable to the 83% survival recorded by Baker (1955, 1954) and above 90% reported by Spurlock (1943). Since larvae were not found in organs other than the digestive tract

after oral infection, it was concluded that N. dubius has no tendancy to migrate from the digestive tract. This would be consistent with a Trichostrongylidae-type of life cycle rather than Nippostrongylus braziliensis type.

N. braziliensis is the only other Heligmosomid whose migratory route has been studied in any detail. Schwartz and Alicata (1934) and Yokogawa (1922) reported success in infecting rats with N. braziliensis by the oral route; but lung migration preceded establishment in the small intestine, and the percentage of infection was low. Simaren (1964) compared the percentage of survival of N. braziliensis larvae after different routes of infection. He found that about 14% of the larvae survived after oral infection, whereas 69% survived after subcutaneous injections. The behavior of N. braziliensis after oral infection differs greatly from that of N. dubius in that they still undergo a lung migration before becoming established in the small intestine.

When the infective larvae of N. dubius were injected into the tail vein, it appeared as though most of them were carried to the heart and lungs via the inferior vena cava and pulmonary artery. Since no larvae were found in the liver and kidneys, all organs of large blood flow and extentive capillary beds, it was presumed that nearly all of the larvae were retained in the lungs. The larvae migrated from the lungs into the trachea, esophagus, and

stomach in the greatest numbers from about 24 to 48 hours after injection. Once the larvae arrived at the small intestine the pattern of development was similar to that of oral infection, however, only about 40% of the larvae could be recovered as adults. The reasons for the lower percentage of adults could not be ascertained. The recoveries of adults were about the same whether sheathed or exsheathed larvae were employed. Either the sheaths did not inhibit this migration or they exsheathed somewhere in the process of migration. It is significant that N. dubius larvae which are adapted for early development in the intestine are also able to undergo a lung to digestive tract migration. The time required for migration from the lungs was comparable to that of N. braziliensis (Twohy, 1956), where most of the larvae leave the lungs within 50 hours after the subcutaneous injection of larvae. Simaren (1964) found that 57% of the infective larvae of N. braziliensis injected into the tail veins of rats survived to adults. This is comparable to the 41% survival found for N. dubius by the same route in this investigation. The infective larvae of N. dubius appear similar to those N. braziliensis in their ability to migrate from the lungs. It could not be determined if the lung-digestive tract migration was due to a passive response in which the capillaries rupture, the larvae enter the respiratory tract and are carried upward by the ciliary current or to a basic behavior pattern of both N. braziliensis and N. dubius.

The results of larval survival in the skin after subcutaneous injection confirms Lepak's (1962) observations, except that more lateral migration was noted and some worms were found 1 inch or farther away from the site of injection. The infective larvae of N. braziliensis, in contrast to those of N. dubius do not remain in the subcutaneous tissues for any length of time after injection or penetration. Twohy (1956) found that most of the larvae of N. braziliensis had migrated from the skin by 24 hours and that none remained longer then 48 hours. The random movement of the infective larvae of N. dubius in the subcutaneous tissues indicated that these larvae did not respond to the stimuli which direct the larvae of N. braziliensis to enter the circulatory system.

The recovery of adults from the small intestine after infective larvae of N. dubius were exposed to the skin of mice was no lower than the recovery from subcutaneous injections. Care was exercised to prevent ingestion of larvae by the mouse. Since a few larvae were found in the subcutaneous tissues of mice exposed to infection by skin penetration, it seemed probable that a minor percentage of the infective larvae have the ability to penetrate the skin. Too few larvae survived to determine the pathway of their subsequent migration. If larvae reached the intestine only as a result of random migration, it would seem that more larvae would have accidentally attained the intestine to develop to adults after subcutaneous injections than skin

penetration because of the greater number in the skin.

The skin is obviously a barrier which can not be penetrated by most larvae. Twohy (1956) found the percentage of infection by N. braziliensis was lower after skin penetration than after subcutaneous injection. The low recoveries from the subcutaneous tissues after cutaneous exposure indicates that it is a greater barrier for N. dubius.

Intraperitoneal injection of N. dubius larvae did not lead to an intestinal infection. Only a few larvae were recovered after injection into this site. This made it seem doubtful that subcutaneously injected larvae reached the intestine via the peritoneal cavity.

Bracket and Bliznick (1949) injected N. braziliensis larvae into the peritoneal cavity of rats and reported recoveries of adults that ranged from 19-35% of the larvae inoculated. From a similar experiment Simaren (1964) obtained 45% of the N. braziliensis larvae as adults. He also showed that after being injected into the peritoneum the larvae migrate through the lungs to reach the digestive tract. It seems that the peritoneal route of infection reduces the survival of both N. braziliensis and N. dubius.

CONCLUSIONS

It has been determined in this investigation that the infective larvae of N. dubius lack many of the abilities necessary to infect by a cutaneous route. Most of the larvae can not penetrate the skin of the host but if injected into the subcutaneous layer can undergo migration. This migration is not directed towards the circulatory system and thus the larvae were not carried to the lungs. The larvae were able to migrate from the lungs to the digestive tract if artificially introduced into the circulatory system.

A small percentage of the infective larvae possessed abilities similar to the infective larvae of N. braziliensis and can penetrate the skin and migrate from the subcutaneous tissues to the small intestine and develop to adult worms, but the route of migration was not determined.

SUMMARY

1. Pepsin digestion of tissues and organs was found to give low recoveries of third and early fourth stage larvae, but the efficiency increased as the larvae developed to adults.

2. Larvae do not migrate outside of the small intestine after oral infection.

3. When sheathed or exsheathed infective larvae were injected intravenously, most of the larvae stopped in the lungs and about 40% of the larvae were able to migrate from the lungs to the digestive tract via the trachea and esophagus.

4. Most of the larvae recovered after subcutaneous injections underwent lateral migration but only a small percentage of the larvae were able to migrate to the small intestine.

5. Larvae injected into the peritoneal cavity did not survive and only a few larvae were recovered.

6. About 3% of the infective larvae exposed to the shaved abdomen of mice were able to penetrate the skin and migrate to the small intestine.

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