Early Development of and Pathology Associated with Strongylus edentatus

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ABSTRACT

Pony foals inoculated with infective Strongylus edentatus larvae were monitored for clinical signs and selected blood changes and were examined at necropsy from two to 56 days postinfection. Larvae penetrated the intestine and reached the liver intravenously before 40 hours postinfection. Occasional thrombi and larval tracks associated with the intima of cecal and colic veins suggested aberrant paths. Larvae in the liver doubled in width between seven and 15 days postinfection and a sudden increment in circulating eosinophils occurred between 11 and 15 days. These changes were probably associated with the third molt. At 30 days fourth stage larvae were migrating in the liver; at 42 days they were present in the hepatorenal ligament.

White foci were observed in the liver from two to 56 days. They contained mononuclear cells and eosinophils and later necrotic cores of eosinophils. By one month foci were overshadowed by tortuous tracks of migrating larvae. Aberrant larvae in the lungs were confined in granulomas. Massive granulomas in the wall of the cecum and colon contained small larvae which were probably inhibited by antibody associated with the third molt. Severe disruption of omental architecture and adhesions involving the intestine occurred several weeks after infection.

RÉSUMÉ

Après avoir administré des larves infectantes de Strongylus edentatus à des jeunes poneys, les auteurs surveillèrent l'apparation de signe cliniques et d'altérations sanguines; ils effectuèrent la nécropsie de ces poneys, de deux à 56 jours après l'administration des larves. Ces dernières atteignirent le foie par la veine porte en moins de 40 heures. La présence de quelques caillots sanguins et sillons larvaires dans l'intima des veines du caecum et du côlon firent penser à des migrations aberrantes. Les dimensions des larves qui atteignirent le foie doublèrent en l'espace de sept à 15 jours. On décela une éosinophilie soudaine entre le 11e et le 15e jour après l'administration des larves. Ces changements accompagnaient probablement la troisième mue. Au bout de 30 jours, les larves du quatrième stade effectuaient leur migration au foie; au bout de 42 jours, on en retrouvait dans le ligament hépato-rénal.

On décela la présence de petits foyers blanchâtres hépatiques, de deux à 56 jours après l'administration des larves. Ils contenaient des cellules mononucléaires et des éosinophiles; un peu plus tard, leur partie centrale contenait des éosinophiles en nécrose. Au bout d'un mois, ces foyers devinrent éclipsés par des sillons tortueux de larves en migration. Aux poumons, les larves aberrantes se trouvaient emprisonnées dans des granulomes. Les granulomes volumineux de la paroi du caecum et du côlon recelaient de petites larves probablement inhibées par les anticorps élaborés lors de la troisième mue. Une désorganisation importante de l'architecture de l'épiploon et des adhérences intestinales se produisirent plusieurs semaines après l'administration des larves.

INTRODUCTION

The migratory strongylines, *Strongylus edentatus, S. equinus* and *S. vulgaris* are the most harmful of all nematodes in horses. Each of these species migrates extensively

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through various organs during their long periods of development before finally establishing as adults in the cecum and colon. The association of *S. vulgaris* with thrombosis and lesions of the walls of arteries, especially the cranial mesenteric artery, is well known (5, 7, 15, 16, 18, 19). *S. vulgaris* has a prepatent period of about six and one half months and experimentally is the most extensively studied of the three species (1, 2, 4, 5, 6, 24). The development of *S. equinus* has been investigated by Wetzel (23, 24) and Enigk (5). It becomes patent at about eight and one half months after infection. During its migratory phase it induces nodules in the serosa of the large intestine and invades the liver and pancreas. It may reach the kidney or other organs before returning to the gut (3).

Early observations on *S. edentatus* were made by Glage (8) and Martin (12). These authors recorded lesions and larvae in the retroperitoneal fat of the flank of foals and adult horses. Of 426 horses examined Martin (12) found the incidence of this strongyle in the abdominal wall to be over 32%. From his experimental infections Wetzel (25) determined that *S. edentatus* reached maturity in 11 months. Later Wetzel and Kersten (27) studied the early development of this strongyle in donkey foals. They found that the molt to the fourth stage occurred in the liver and they recorded pathological changes associated with *S. edentatus* in this organ.

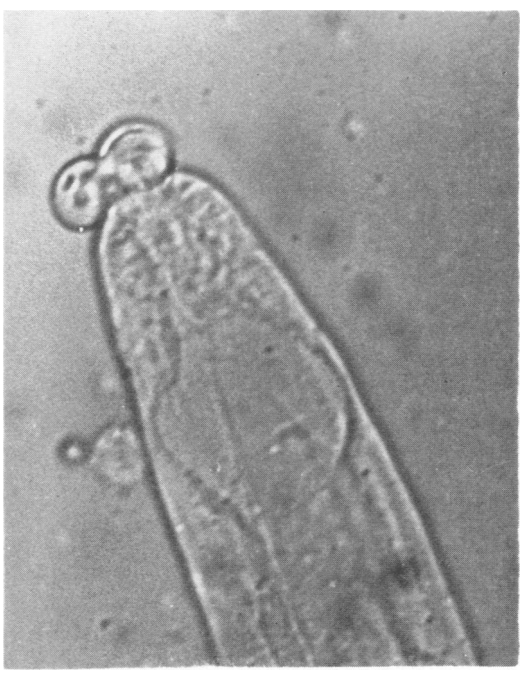

Fig. 1. Third stage S. edentatus **larva recovered from liver 40 hours postinfection. Three vesicular structures, two of which are shown, protrude from the anterior end. X125.**

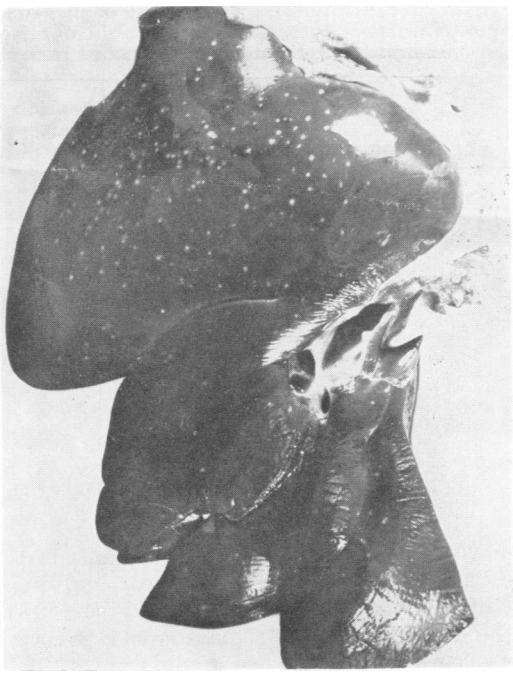

Fig. 2. Prominent white foci on the parietal surface of the liver at seven days postinfection.

In a recent survey carried out by Slocombe and McCraw (22) it was found that *S. edentatus* occurred in over 93% of horses in Ontario five years or more of age. Moreover, this nematode was the most abundant of the three *Strongylus* species and accounted for 55% of these strongyles. In view of the more limited studies on *S. edentatus* it is our purpose to examine closely the development of this common nematode and to study the clinical and pathological changes following infection with it. This paper records observations during the first eight weeks of infection.

MATERIALS AND METHODS

Twelve pony foals, mainly Shetland, were obtained within 12 to 24 hours after birth and reared in isolation on raised wire-bottom cages (Table I). Foals were bottle-fed either Foal-lac[1] or a formula consisting of

[1]Borden Chemical, Borden Inc., Norfolk, Virginia.

TABLE I. Experimental Strongylus edentatus. Age of Ponies at Infection, Number of Infective Larvae given and Duration of Infection

Pony no	Age at infection	No of infective larvae	Duration of infection
1	38 days	50000	40 hours
2	173 days	15000	2 days
3	87 days	15000	2 days
4	223 days	15000	3 days
5	75 days	15000	4 days
6	80 days	45000	7 days
7	65 days	15000	15 days
8	296 days	10000	27 days
9	71 days	15000	30 days
10	177 days	15000	34 days
11	61 days	15000	42 days
12	53 days	15000	56 days

powdered skim milk, powdered whole milk, corn oil, dextrose, cod liver oil and lime water. The milk replacer was given until the foals were three months old. At two weeks of age a mixture of nine parts Complete Horse Feed[2] and one part linseed oil meal was given *ad lib*. This mixture was the only ration given to the foals after the milk replacer was withdrawn.

Adult *S. edentatus* were collected from the cecum and colon of naturally infected horses. Female worms were chopped finely with a scalpel and mixed thoroughly in damp sterile sheep feces and placed in a syracuse watch glass. This culture was then placed in a petri dish to which was added 0.85% saline to approximately 6 mm from the top of the watch glass. The petri dish was covered and the culture incubated at 26°C for nine to 12 days. Infective larvae were harvested from the saline in the petri dish and stored in 0.85% saline at 6°C until required.

Larvae for inoculation into foals were counted by a dilution technique. Experimental animals were dosed with a stomach tube inserted through the nostril. For smaller foals (up to about 75 lbs) at 75 cm 18 F rubber Rüsch catheter was used and for larger foals at 75 cm 22 F rubber colt catheter was utilized. Each catheter was fitted with an adapter which allowed snug attachment of a 35 ml disposable syringe. All dosing equipment was silicone-coated[3]

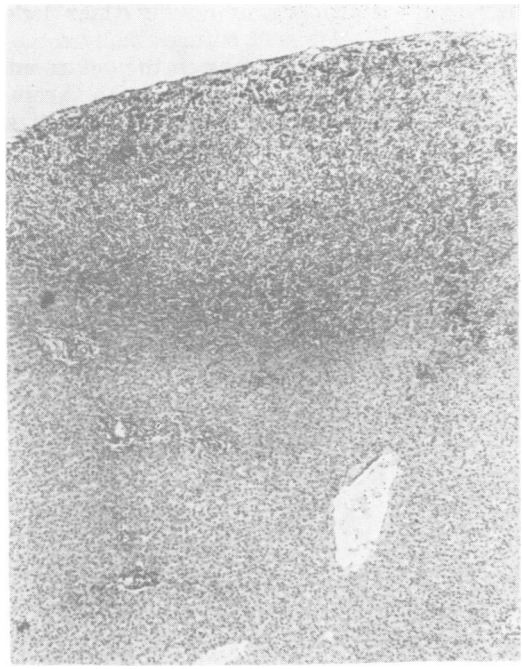

Fig. 3. Section of white focus without a core observed in the liver on days 4 and 7 postinfection; these foci consisted of aggregations of mononuclear cells and eosinophils. X400.

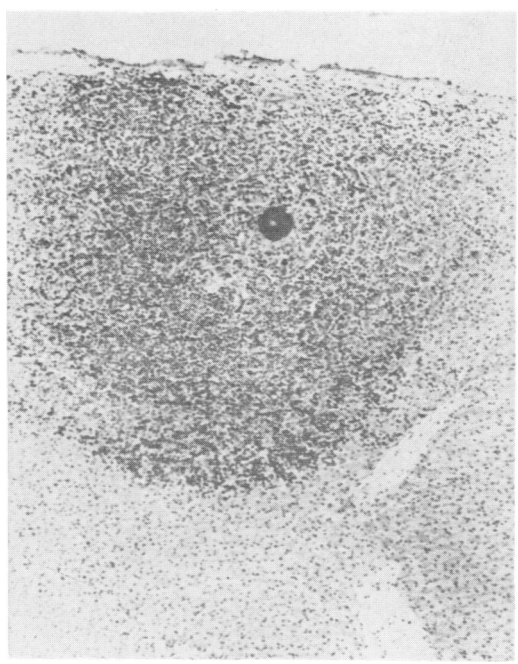

Fig. 4. Section of a white focus with a core of necrotic eosinophils surrounded by mononuclear cells and normal eosinophils; this type of white focus was first observed in the liver on day 7 postinfection. X400.

[2]Master Feeds, Division of Maple Leaf Mills Limited, Toronto, Ontario.

[3]Siliclad, Clay-Adams Inc., New York, New York.

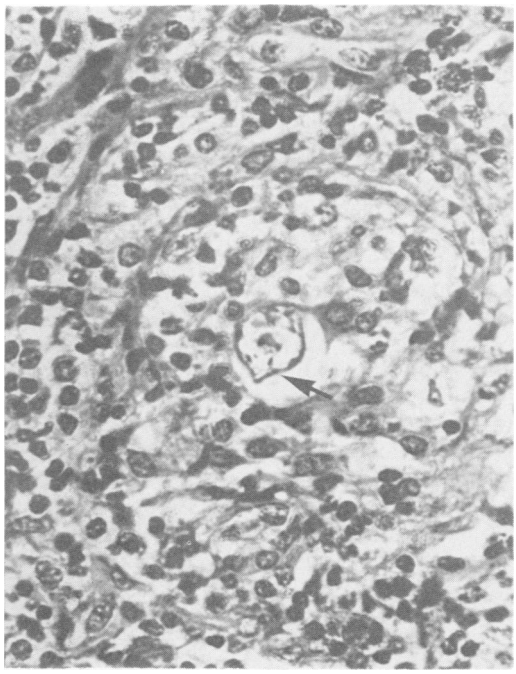

Fig. 5. Cross section of third stage S. edentatus larva (arrow) in the liver seven days postinfection. Larvae were most often observed at this time in white foci with cores of necrotic eosionphils. X1600.

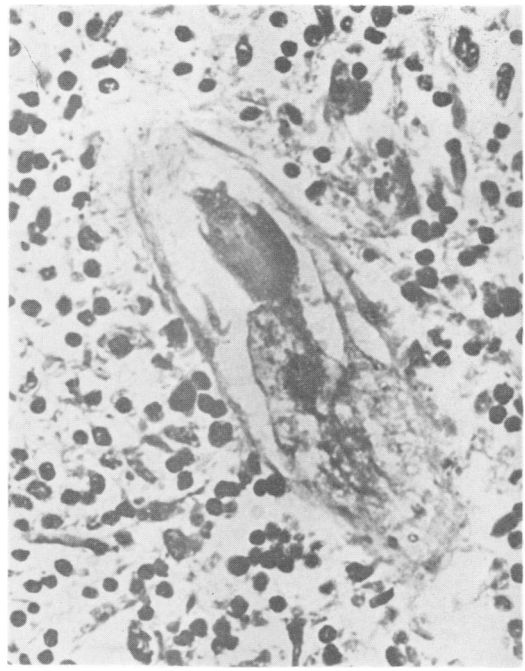

Fig. 6. S. edentatus larva in the liver 15 days postinfection. By this time larvae had grown considerably, and measured 43 to 46 μ in diameter, double that of day 7 postinfection. X1500.

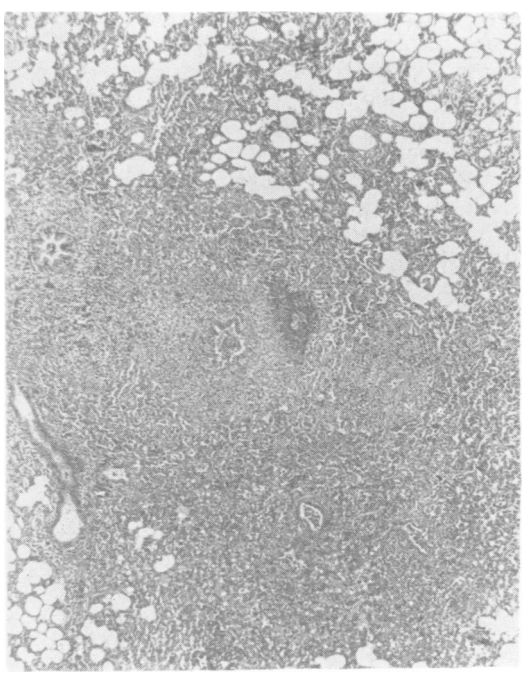

Fig. 7. Granuloma in the diaphragmatic region of the right lung 15 days postinfection. A centrally located accumulation of necrotic eosinophils is present. X100.

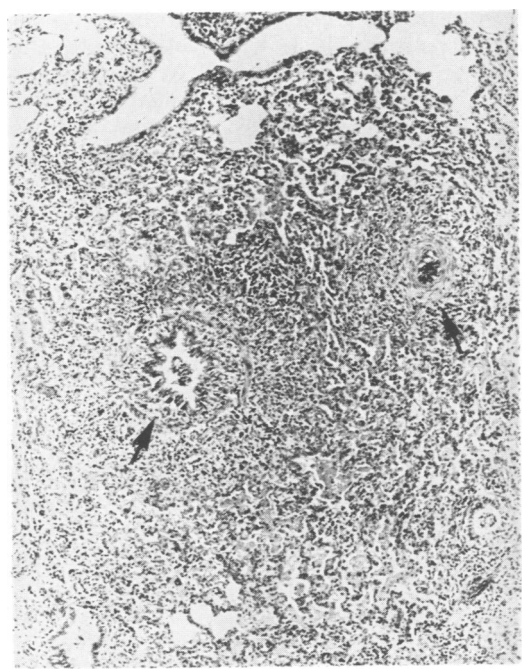

Fig. 8. Accumulations of mononuclear cells around an arteriole and an adjacent bronchiole (arrows) 15 days postinfection. Note fibrin within and infiltration of alveoli near arteriole and bronchiole. X160.

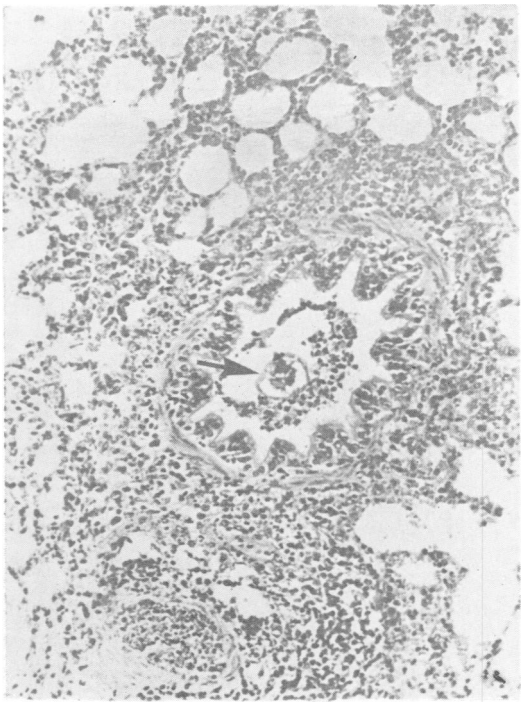

Fig. 9. S. edentatus larva (arrow) in a bronchiole at 15 days postinfection. X400.

to prevent larvae from adhering to surfaces. Larvae were suspended in 35 to 50 ml of saline which was poured into the syringe. This was followed by a thorough flushing of the dosing equipment with water. Information on the infection of foals is given in Table I. They were generally infected with 15000 ± 6% *S. edentatus* larvae and were examined from two to 56 days postinfection.

Blood parameters monitored included: Hb%, PCV%, RBC per cu mm, WBC per cu mm and total circulating eosinophils per cu mm. Circulating eosinophils were counted in a double-ruled Fuchs-Rosenthal chamber following the method of Randolph (20). Infected foals were routinely observed for clinical changes.

At necropsy the following organs and structures were examined for *S. edentatus*: the small and large intestine and associated mesenteries and lymph nodes, the liver and its associated ligaments and lymph nodes, pancreas, spleen, kidneys, adrenals, diaphragm, lungs, abdominal wall and brain. In addition to peritoneal fluid and the heart, the following blood vessels were examined for lesions and *S. edentatus* larvae: aorta and posterior vena cava, the cranial mesentric, pancreatico-duodenal, splenic, dorsal and ventral colic, lateral and medial cecal, ileal, renal and internal and external iliac arteries and veins.

Major organs and structures were processed for recovery of *S. edentatus* larvae. Up to one half of the intestine was fixed to wood discs and placed mucosal surface down in Baermann preparations with 15 cm wide funnels containing 1% pepsin hydrochloride. These intestinal segments were held in place with fibreglass screening (18 mesh/inch), incubated at 37°C and examined after four and 18 hours. Up to the end of the second day postinfection the entire ileum, cecum, dorsal and ventral colon were processed along with selected segments of the remainder of the intestine.

The entire liver was examined for larvae except on days 7 and 56 postinfection when only half the liver was examined. It was found best to cut liver finely with scissors and place 50 to 60 gm in a Baermann apparatus containing normal saline heated to 40°C. Baermann samples were usually drawn after four hours (before clouding occurred) and after a further 12 to 16 hours. Up to 30 days postinfection liver samples were placed in funnels on one layer of gauze supported by a fibreglass screen 11 cm in diameter or on a screen beneath which were two layers of Kimwipe[4], one layer of gauze and another screen. With the latter arrangement both the upper surface of the Kimwipe and the usual Baermann samples were examined. Where smaller larvae were present the fluid contents were also examined and the liver samples were further processed by flushing through a mesh no. 50 and 200 screen[5]. With livers examined at two days postinfection, in addition to Baermann preparations, chopped 50 gm samples were added to 100 ml of normal saline and 50 ml of water, stirred and filtered through a coarse sieve and the filtrate centrifuged and observed for larvae.

Baermann preparations of the pancreas and spleen were the same as those for liver. Renal tissue was cut with scissors or ground briefly in a Waring Blendor and placed in a gauze-screen-saline Baermann preparation. The capsule of the kidney was examined separately with a stereoscopic microscope. Pulmonary tissue was also cut with scissors

[4]Kimwipe, 900-L Kimberley Clark Industrial Paper Products, Toronto, Canada.

[5]Endecotts, Limited, London, England.

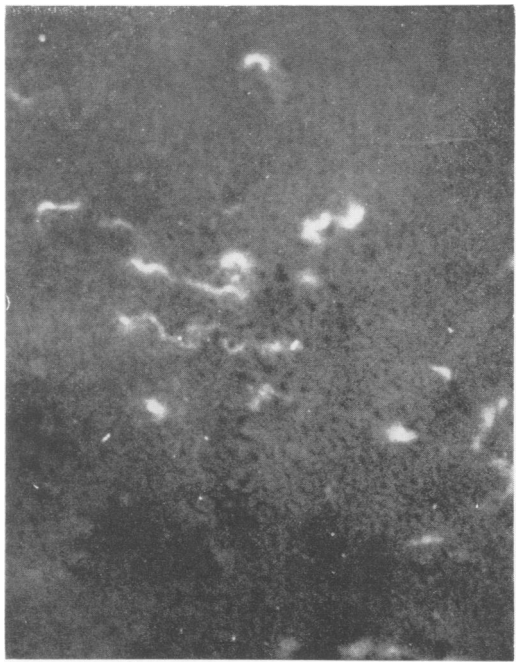

Fig. 10. White tortuous tracks observed on the surface of the liver at 34 days postinfection.

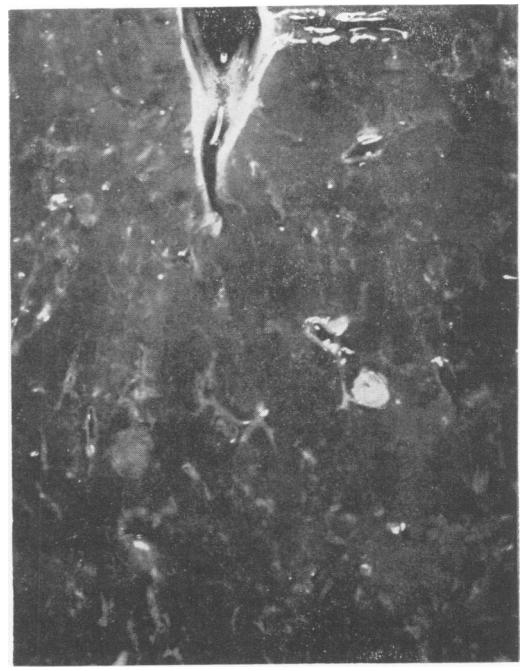

Fig. 11. White tortuous tracks observed in the parenchyma of the liver at 30 days postinfection.

and then placed in normal saline. Finely sliced nodes were placed in 1% pepsin or saline and both lungs and nodes were set in a gauze-screen preparation. Although occasionally left at room temperature, most Baermann preparations were incubated at 37°C and read after 18 hours (except liver).

Various organs and tissues were fixed in 10% formalin (the intestine generally in Bouins) sectioned and stained with hematoxylin and eosin. Major blood vessels were also stained with aldehyde fuchsin.

RESULTS

Days 2 and 3 Postinfection

From one pony examined 40 hours after infection eighteen *S. edentatus* third stage larvae were recovered from the liver. These were distinguished by the formation of three vesicular structures protruding from the anterior end (Fig. 1). Gross lesions were first observed two days postinfection (pony 3) and these consisted of seven barely discernible white foci present on the parietal surface of the liver. The peritoneal cavity of this pony contained an abnormal amount of cloudy light brown fluid. No larvae were recovered. No lesions or larvae were found in a third pony (no. 2) killed toward the end of the second day after infection. Pony 4, killed on day 3, was similarly negative. All other organs were normal.

Day 4 Postinfection

Many white foci 2 to 3 mm in diameter were present on the surface of the liver. A few were seen in the parenchyma. On the parietal surface about 80 foci were counted on the right lobe and 25 on each of the middle and left lobes. About 40 evenly distributed focal lesions were also observed on the visceral surface. Microscopically these white foci consisted of loose aggregations of mononuclear cells and eosinophils. They had poorly defined borders and were often observed near portal areas. One third stage larva was recovered from the liver. Some atelectasis was evident in the apical region of the lungs. The remaining organs were normal.

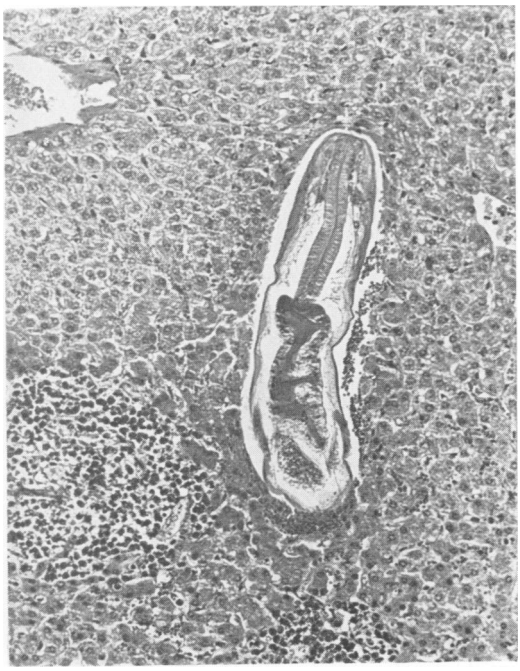

Fig. 12. Fourth stage S. edentatus larva in the liver at 30 days postinfection. X400.

DAY 7 POSTINFECTION

By this time white foci were more prominent on the liver (Fig. 2) and from these five third stage larvae were dissected. Two types of hepatic foci which were more discrete than any seen previously were observed: those with a minute, dense white core and those without a core. No larvae were recovered by Baermann or flushing techniques. In sections, foci without a core were similar to those observed on day 4 (Fig. 3). Those with a core contained in addition to mononuclear cells and eosinophils a central mass of necrotic eosinophils often enclosing remnants of larvae (Fig. 4). Both types of foci had ill-defined borders generally surrounded by eosinophils. In foci with cores larvae were often observed (Fig. 5) and these ranged from 19 to 24 μ in diameter. All other organs were normal.

DAY 15 POSTINFECTIVN

The liver had the same pathological changes as on day 7. However, larvae observed in foci had grown considerably in the interval and measured 43 to 46 μ in diameter (Fig. 6).

Although the cecum and colon were normal grossly, one larva 43 μ in diameter was found in a lymph nodule in the submucosa of the right ventral colon. For the first time, lesions other than atelectasis were noted in the lungs. These consisted of small red foci 3 to 5 mm in diameter, four were present on the lateral surface of the diaphragmatic region of the right lung and one in the same region of the left. In sections these were found to be granulomas often containing tracks surrounded by necrotic eosinophils (Fig. 7). The alveolar architecture peripheral to a granuloma was often severely disrupted with hemorrhage, fibrin and proteinaceous fluid present. Accumulations of mononuclear cells around an arteriole and an adjacent bronchiole was a frequent observation within or near a granuloma (Fig. 8). One larva, 45 μ in diameter, was found in a bronchiole. Both its internal structure and body wall appeared degenerated (Fig. 9).

DAYS 27, 30 AND 34 POSTINFECTION

By this time, in addition to foci, white tortuous tracks were present on the surface of and within the liver (Figs. 10 and 11). These were 10 to 12 mm long and about 1

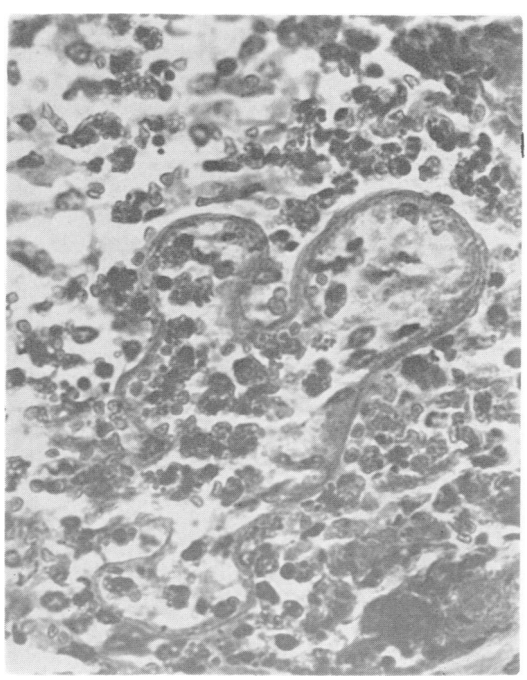

Fig. 13. Remnants of an S. edentatus larva in the liver at 30 days postinfection. X1600.

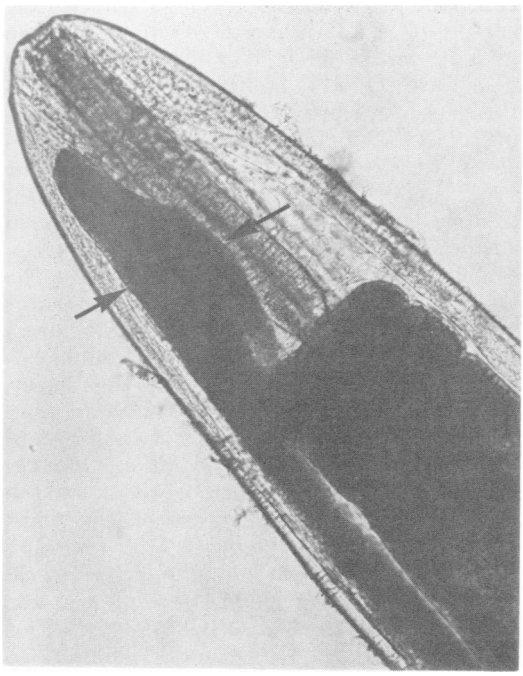

Fig. 14. Fourth stage S. edentatus **larva recovered from the liver at 30 days postinfection. Note paired structures (arrows) evidently glands, within the anterior end of the larva. X100.**

mm wide and many tracks seen on the surface had a distinct fine red border. Tracks were more prevalent than white foci and both were more numerous on the parietal than on the visceral surface of the liver. Tracks were especially abundant in the hepatic parenchyma of pony 9, killed 30 days postinfection.

White foci in the liver were similar to those seen on day 7. However the composition of tracks was variable. Some were composed of a mixture of mononuclear cells and eosinophils, while others contained a central mass of necrotic eosinophils surrounded by mononuclear cells peripheral to which was a ring of normal eosinophils. Giant cells surrounding eosinophil granules were commonly observed. The occasional track composed entirely of eosinophils was also present. Hemorrhagic areas were found in sections of liver but hemorrhage was definitely not an outstanding microscopic feature.

Both living and dead or remnants of larvae were seen in sections of liver (Figs. 12 and 13). A total of 99 living fourth stage larvae were recovered from the liver on day 30. These larvae were 4 or 5 mm long (Fig. 14). There was little evidence of a buccal cavity and the esophagus was short and straight. A pair of long dense structures, evidently glands, extended from the anterior end of the esophagus to beyond the middle of the larva. In sections these glands were deeply eosinophilic (Fig. 15).

Lesions in the intestine were confined to the cecum and colon. These consisted of nodules, many of which were hemorrhagic, on the serosal surface. The nodules were 2 to 8 mm in diameter and 3 to 5 mm in height (Fig. 16). In pony 10, examined 34 days after infection, they were more concentrated in the cecum than in the colon and accumulated near the apex which was very thick and firm. In the remainder of the large intestine nodules were present in greatest numbers in the right ventral colon (Fig. 16). Larger nodules were either palpable or visible from the mucosal surface of the right ventral colon. Lymph nodes on the serosal surface of the cecum and colon were generally enlarged and hard and many had hemorrhagic centers. In ponies 8 and 9 (27 and 30 days postinfection) these nodes formed a continuous chain especially at the base of the cecum and the origin of the right ventral colon. Raised rough areas 1 to 1.5 mm in size

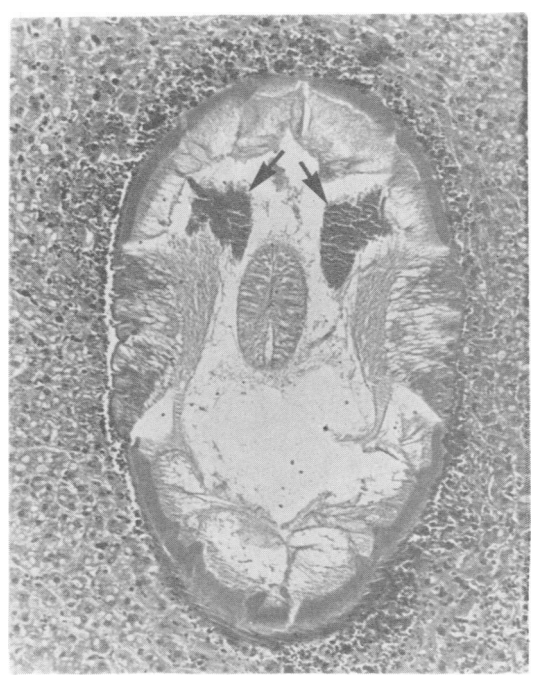

Fig. 15. Cross section of a fourth stage S. edentatus **larva in the liver at 34 days postinfection. Paired structures seen in Fig. 14 are evident in the section (arrows). X400.**

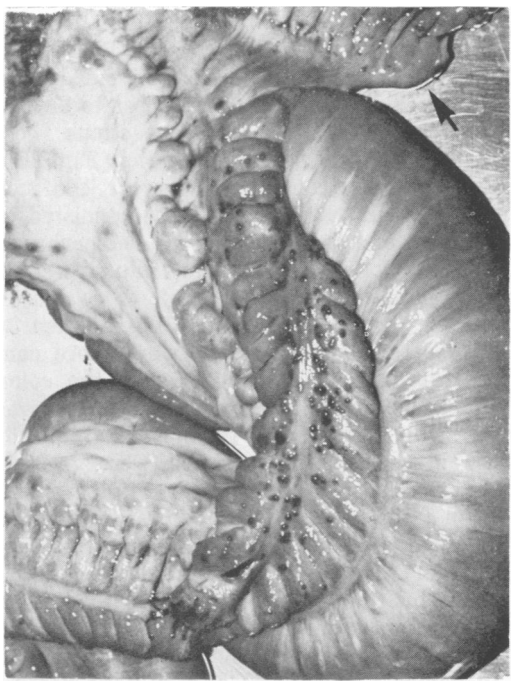

Fig. 16. Nodules on the cecum (arrow) and ventral colon at 34 days postinfection.

were present on the intimal surface of the medial cecal and ventral colic veins in pony 9. These areas were irregularly distributed. There were no changes noted in the remainder of the intestine.

The mucosa of the cecum contained large numbers of eosinophils which were widely distributed. In the submucosa a few foci or dense accumulations of eosinophils containing remnants of larvae were observed. Some foci contained tracks of larvae and the greatest diameter of these tracks was about 28 μ. Other than infiltration by eosinophils and mononuclear cells no lesions were noted in the tunica muscularis. By far the greatest changes were seen on the serosal surface of the cecum. Here the lymph nodes were very reactive and there was often an intense infiltration of eosinophils and a fibrous reaction between lymphoid aggregations. Isolated accumulations of giant cells were common. Many sites of necrotic eosinophils surrounded by an epithelioid reaction or giant cells were present (Fig. 17). Tracks, usually with remnants of larvae were commonly seen and these tracks varied from 40 to 70 μ in diameter with most in the 40 to 50 μ range. The intima of arterioles in the serosa and to a lesser extent in the submucosa was very reactive with intimal bodies readily observed (Fig. 18). Raised rough areas seen grossly on the intima of the medial cecal vein were found to be organized thrombi in the centers of which were cores of mostly necrotic eosinophils (Fig. 19). Similar lesions were present on the intima of the ventral colic vein. The wall of the ventral colic vein was also heavily infiltrated with eosinophils and tracks were present immediately beneath the intima and within the wall of the vessel (Fig. 20). In the right ventral colon larvae or tracks of larvae were found at the junction of the submucosa and tunica muscularis, within the muscularis and within the serosa. The diameters of larvae seen in sections of the right ventral colon varied from 50 to 62 μ. One fourth stage larva was recovered from the nodes on the serosal surface of the right ventral colon.

Two nodules were noted on the lateral surface of the diaphragmatic region of the lung of pony 10. These contained foci of esinophils along with tracks similar to those seen in the cecum and colon. The greatest diameter of these tracks was 45 μ. One larva and a portion of another were recovered from the lung of pony 9.

Day 42 Postinfection

Fibrous adhesions involving the greater omentum, the small intestine and the cecum were present (Fig. 21). Disruption of the omental architecture was severe and the structure was largely reduced to a series of strands. An abnormal amount of amber-colored cloudy peritoneal fluid was present. Lymph nodes along the portal vein were prominent and those at the portal fissure were greatly enlarged, two being over 2.5 cm in diameter.

The distal portion of the cecum was thickened and very firm with numerous nodules, several of which were 3 cm or more in diameter. The principal microscopic changes found in the cecum were on the serosal surface which was exceedingly fibrotic and infiltrated by massive numbers of eosinophils. Granulomas riddled with tracks 40 to 50 μ in diameter were very numerous.

Although both tracks and white foci were found on the surface and in the parenchyma of the liver the latter lesions were more numerous and certainly more prominent. These foci were also frequently

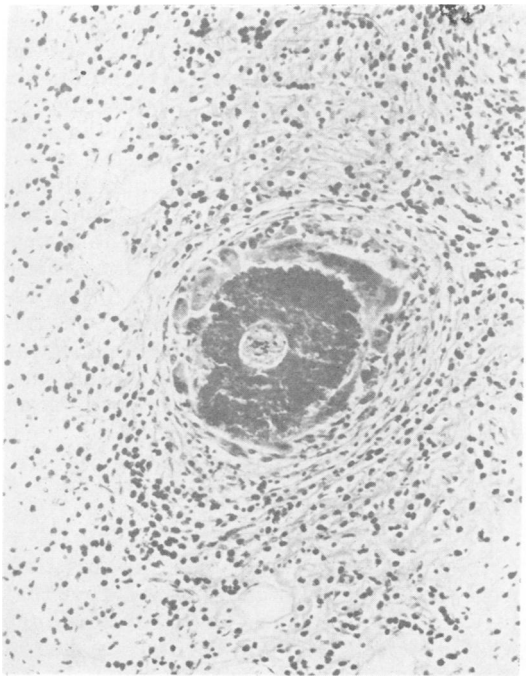

Fig. 17. Remnants of an S. edentatus larva surrounded by necrotic eosinophils and giant cells in the cecum at 30 days postinfection. X400.

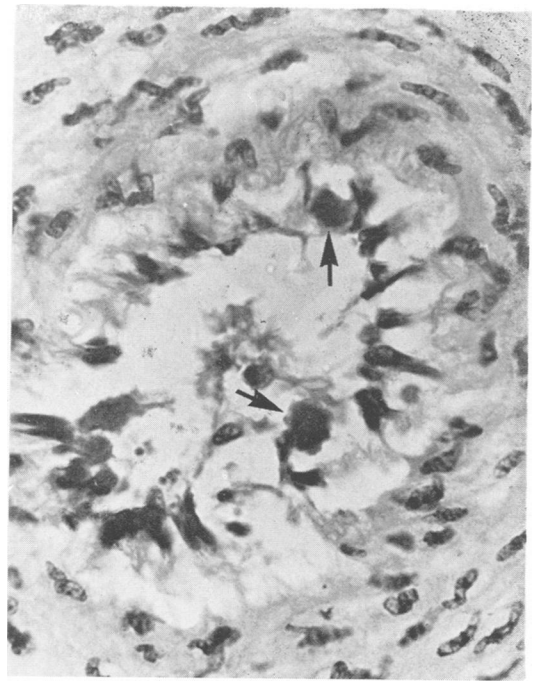

Fig. 13. Reactive intima of an arteriole in the submucosa of the cecum at 30 days postinfection. Note "intimal bodies" (arrows). X1600.

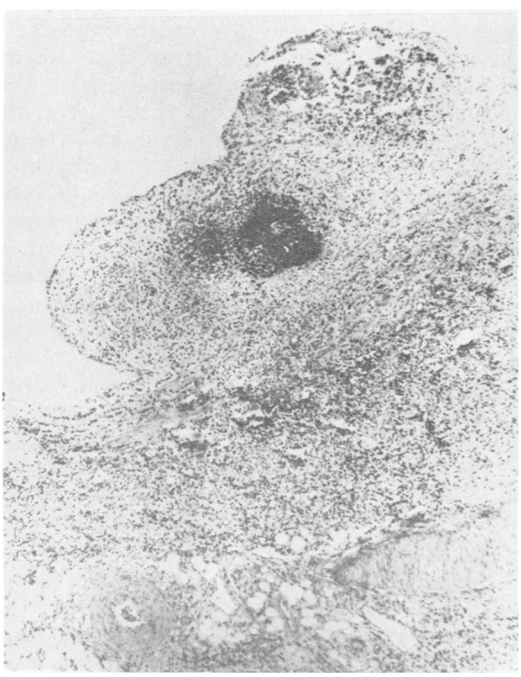

Fig. 19. Small thrombus with a core of necrotic eosinophils in the medial cecal vein at 30 days postinfection. X400.

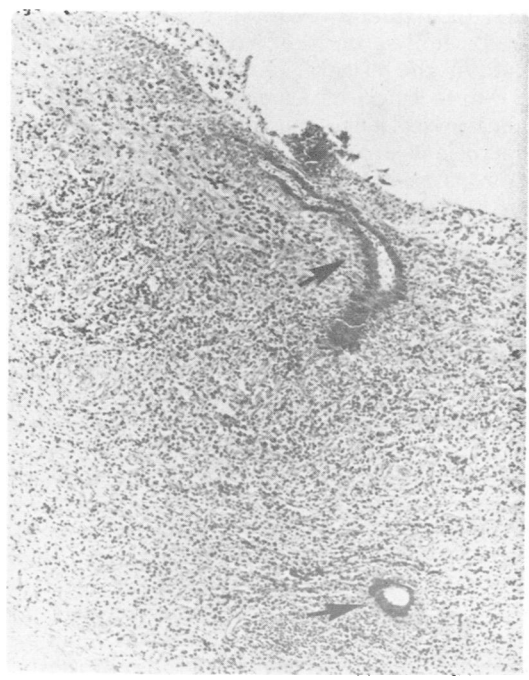

Fig. 20. Tracks (arrows) beneath the intima and within the wall of the ventral colic vein at 30 days postinfection. X160.

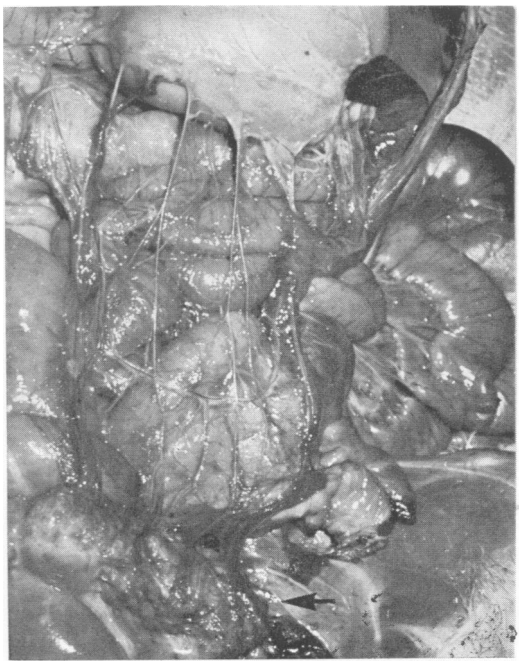

Fig. 21. Adhesions involving the greater omentum, small intestine and cecum (arrow) at 42 days postinfection.

larger and more often raised than those observed previously. Many were up to 3 mm in diameter. A total of 480 white foci was counted on the parietal surface and 247 on the visceral surface of the liver.

Three types of lesions were observed in hepatic sections. One consisted of a core of necrotic eosinophils surrounded by a strong fibrous reaction peripheral to which was a ring of mononuclear cells and eosinophils. This lesion accounted for the raised white foci so prominent on the surface of the liver (Fig. 22). A second consisted of foci or tracks containing a mixture of neutrophils and mononuclear cells interspersed with eosinophils (Fig. 23). The third consisted of tortuous necrotic tracks or foci infiltrated primarily with neutrophils along with a few eosinophils (Fig. 24). All these lesions were very numerous. Fourth stage larvae were observed in liver sections. In addition, periportal infiltration of eosinophils was very common. Hemorrhage was not a microscopic feature of the liver. Hepatic portal nodes were not reactive and no evidence of migrating larvae was found in these nodes. Seventy-three fourth stage larvae were recovered from the liver. One fourth stage larva was also dissected from the hepatorenal ligament.

Nodules up to 8 mm in diameter were present on the surface of the lungs. Seven were observed on the left lung and six on the right. Each of three dissected had a caseous core. The lungs were otherwise normal except for atelectasis in the apical region. These nodules were found to be composed of several contiguous granulomas which usually contained tracks. Unlike previous granulomas seen in the lung these were characterized by a strong fibrous reaction. The diameter of the tracks was between 45 and 50 μ. Only one track was found to contain remnants of larvae. One fourth stage larva was recovered from the lungs.

Day 56 Postinfection

In general, the lesions seen at this time were similar to those at 42 days except that they were less extreme. The greatest change was observed in the liver which had a scarred and mottled appearance. Although less distinct than at day 42, tracks were nevertheless very numerous and tended to interconnect. There were fewer foci and all but ten of the 89 counted were on the parietal surface. They were also larger and more raised than any seen previously with many up to 5 mm in diameter. There was less fibrosis associated with these foci than at day 42. For the first time small hemorrhagic areas were seen in sections. Large larvae with a maximum diameter of 605 μ were present in the liver and there was usually little or no reaction around them. A total of 116 fourth stage larvae was recovered from the liver and ten were dissected from the hepatorenal ligament.

Five hemorrhagic areas, one of which was approximately 1 cm in diameter, were present beneath the surface of the pancreas. Although many eosinophils were present in these areas no larvae were found. One fourth stage larva was recovered from a Baermann preparation of the right kidney. Eight nodules were observed on the lungs, three of which were on the ventral surface of the cardiac region of each lung.

Clinical Pathological Findings

Following infection no clinical signs attributable to *S. edentatus* were observed and no significant changes were found in Hb, PCV or RBC. In four of six ponies in-

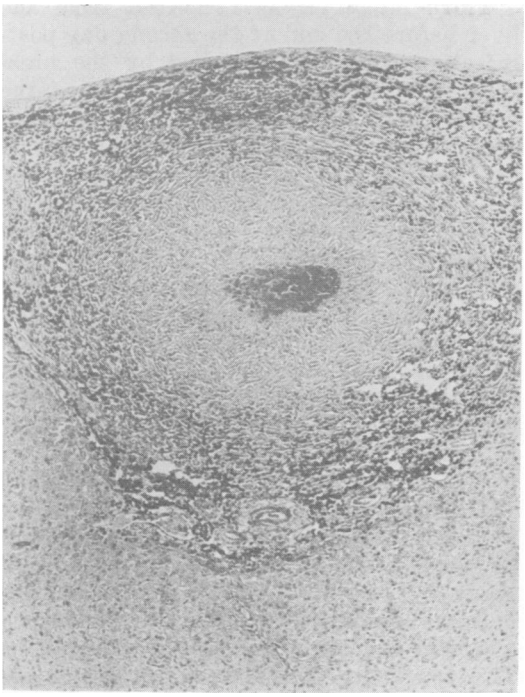

Fig. 22. Strong fibrous reaction surrounding necrotic eosinophils observed in the white foci of the liver at 42 days postinfection. X100.

creases in total eosinophil counts were observed between 11 and 15 days postinfection. In the remaining two, increments were first detected 20 and 27 days after infection. Changes in eosinophil counts of pony 10 are shown in Fig. 25.

DISCUSSION

Although the extent of the intestine invaded by third stage *S. edentatus* larvae was not determined it seems certain that the cecum and right ventral colon are major sites of penetration. Larvae and remnants of larvae were still present in the submucosa, muscularis and serosa of these regions one month after infection.

Following infection larvae rapidly attained the liver. The recovery of third stage larvae from this organ before the end of the second day of infection and the frequent association of lesions with portal regions on the fourth day are consistent with a portal route of migration. The presence

Fig. 23. Tracks containing a mixture of neutrophils and mononuclear cells interspersed with eosinophils observed in the liver at 42 days postinfection. X100.

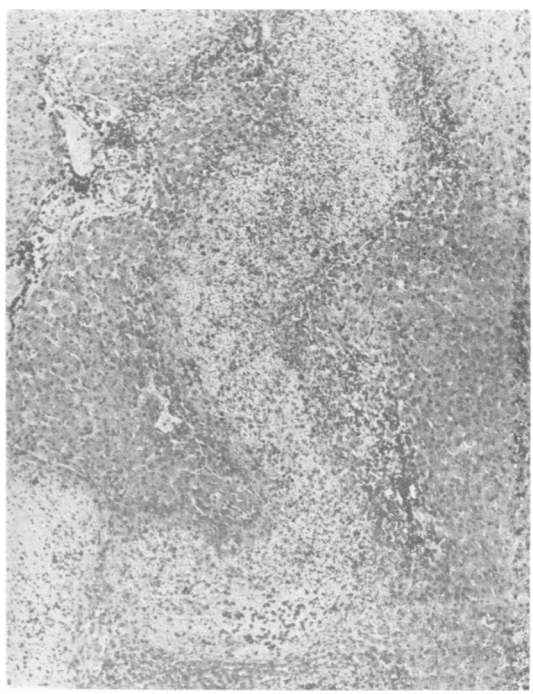

Fig. 24. Tortuous necrotic track in the liver at 42 days postinfection. X100.

of thrombi containing cores of necrotic eosinophils on the intima of the medial cecal vein supports the conclusion that the portal route is probably the common one. These lesions, seen 30 days after infection, presumably resulted from larvae that became closely applied to the vessel wall shortly after infection. Tracks were also present at this time beneath the intima and within the wall of the ventral colic vein but whether *S. edentatus* larvae normally become associated with the walls of veins requires further study. Lesions of the walls of veins were not commonly observed and this fact coupled with the early establishment of larvae in the liver would suggest that *S. edentatus* follows an intravenous route with only aberrant association with the intima or walls of veins. Wetzel and Kersten (27) who examined the livers of foals beginning on the sixth day postinfection also concluded that *S. edentatus* reached the liver via the portal system. At no time were larvae recovered from peritoneal fluid and there was no evidence that this strongyle migrated to the liver through the peritoneal cavity.

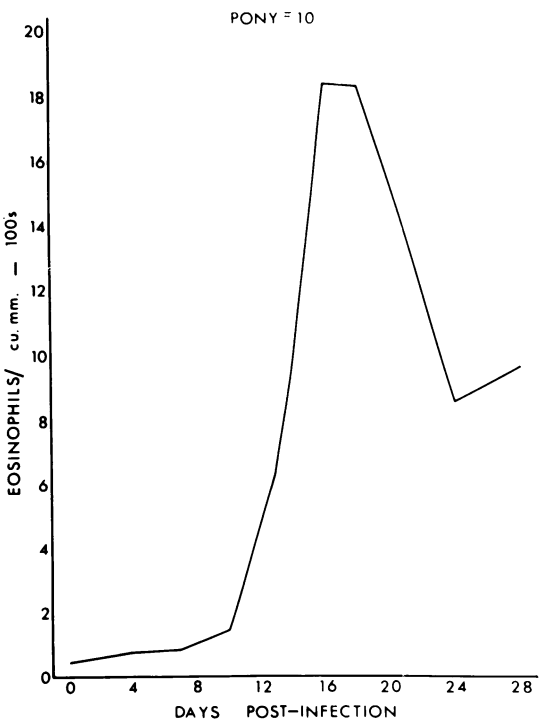

Fig. 25. Changes in eosinophils per cu mm in pony 10 following infection with S. edentatus.

Third stage larvae recovered from the liver before the end of the second day postinfection were distinguished by the presence of three vesicular structures at the anterior end. These were separated by deep grooves and were also observed by Wetzel and Kersten (27). Similar cephalic structures have been described in the third stage larvae of *Strongylus vulgaris* (10) and *Strongylus equinus* (23). Wetzel and Kersten (27) stated that these protuberances were capable of inflation and deflation and they believed that they functioned to assist larvae in their migration through tissues.

Third stage *S. edentatus* larvae were present in the liver up to seven days after infection. In sections these measured 19 to 24 μ in diameter. Wetzel and Kersten (27) reported third stage larvae up to day 11 postinfection and the cephalic structures on these larvae up to day 6. In the present study by 15 days larvae seen in sections had increased to 43 to 46 μ in diameter indicating that the third molt had occurred. This conclusion is further supported by the sudden increment between the 11th and 15th days in total circulating eosinophils which presumably responded to the release of antigens at the time of the third molt. A similar increment in eosinophils occurs about the same time in calves following infection with *Ascaris suum* (9, 13). Wetzel and Kersten (27) concluded that *S. edentatus* larvae molted to the fourth stage between days 11 and 18 and our results in general agree with this conclusion.

The presence of tortuous tracks on the surface of the liver and in the parenchyma one month after infection suggested that the fourth stage larvae were undergoing active migration. Larvae were also readily recovered at this time. Wetzel and Kersten (27) observed fine tracks 4 to 5 mm long in the liver as early as 19 days postinfection. It seems likely therefore, that a migratory phase is initiated by *S. edentatus* larvae very soon after the third molt. The large glands seen in fourth stage larvae around one month after infection are probably cytolytic in function. Previous authors (27) mention only a "ventral gland" in these larvae but our observations revealed that the structure is paired (Figs. 15 and 16). Certainly the ease and rapidity with which fourth stage larvae are able to migrate at this time is indicated by the absence of any reaction around the anterior end (Fig. 13). However, these larvae. which were only slightly active even when

placed in saline at 37°C, are evidently unable to penetrate the capsule of the liver.

One of the more curious aspects of *S. edentatus* development has been the route by which it escapes from the liver. From their studies Wetzel and Kersten (27) assumed that larvae reached the parietal peritoneum via the ligaments of the liver. In the present study *S. edentatus* larvae were found within the hepatorenal ligament as early as 42 days postinfection. Neither at this time nor at 56 days were they observed in other ligaments associated with the liver and it would appear that this ligament is a primary route of migration of *S. edentatus* at least during the early stages of migration. Since the hepatorenal ligament extends to the base of the cecum (21) a path is readily available for larvae to migrate to the intestine.

The earliest lesions that could be related to *S. edentatus* infection were white foci on the surface of the liver. These developed promptly and were evident as early as the second day postinfection. They soon became more distinct and prominent and remained so from the seventh day to several weeks after infection. This change was first brought about by an increase in concentration of mononuclear cells and in addition by the development of white cores which consisted almost exclusively of necrotic eosinophils. Wetzel and Kersten (27) described white nodules between six and 36 days. Although overshadowed by tracks in the liver by about a month after infection, at six weeks these foci were larger, raised, tended to be cream colored and were characterized by a strong fibrous reaction. Giant cells were not associated with white foci and it would appear that this reaction is primarily a response to the prolonged release of antigens from larvae. Why foci were more common on the parietal than on the visceral surface of the liver is unknown.

Tracks, death of larvae and necrosis are evidently a later pathological change of *S. edentatus*-infected livers. Although hemorrhage has been reported as both a gross and microscopic feature of *S. edentatus*-infected livers (27) it was not observed grossly by us and was rarely encountered microscopically.

Larvae of *S. edentatus* evidently reach the lung only aberrantly. Since they were first found on day 15, with the accumulation of mononuclear cells around an arteriole in the lung a frequent observation, it seems clear that a vascular route is followed. Whether they attained the lung as third or fourth stage larvae was not determined. As only one degenerate larva was observed in a bronchiole, it is unlikely that a tracheal migration occurs. However, the presence of tracks in granulomas indicated that a limited migration within the lung takes place. The strong fibrous reaction present around older granulomas (e. g. six weeks after infection) would tend to confirm that *S. edentatus* larvae in the lung are confined there. In their studies Wetzel and Enigk (26) found no gross lesions in the lung associated with *S. edentatus* infection and likewise were unable to detect larvae in the lung.

The massive granulomas found in the cecum and right ventral colon beginning about one month after infection contained many tracks and remnants of larvae. These tracks and all the larvae observed never attained the dimensions of those found in the liver. It is therefore quite possible that these young *S. edentatus* were inhibited in development as a result of antibody induced by larvae which reached the liver and molted to the fourth stage soon after infection. Moreover, larvae may be slower for anatomical reasons in migrating to the liver from the cecum and colon than from the small intestine and this would favor the accumulation of inhibited forms in the lower intestine.

By one month after infection intimal bodies in arterioles of the cecum and right ventral colon were very common. These have been attributed to *Strongylus vulgaris* infection (11) but Montali *et al* (14) found no relation between these structures and *S. vulgaris*.

Severe disruption of omental architecture as well as adhesions involving the intestine occurred after several weeks of *S. edentatus* infection. Unfortunately, bacterial culture was not done. However, sensitization to *S. edentatus* could well be considered as a possible cause. In aged horses pathological changes of the greater omentum such as adhesions and twisted strands are said to be a common occurrence (21) and our observations suggest *Strongylus* infections as one of the major causes of these changes.

The absence of clinical signs in our *S. edentatus*-infected ponies contrasts with the findings of others in the Equidae (17, 25, 27). An eight month old horse foal infected by Wetzel (25) with only 250 *S. edentatus* larvae developed signs of colic

three and eight weeks after infection. The animal stood with its legs extended and was down periodically rolling on the floor. Similar observations have been made by Wetzel and Kersten (27) with donkeys. One developed enteritis three days after infection with 25,000 larvae. Another, given 10,000 larvae, was inactive and off feed between six and 11 days postinfection and a third gradually weakened and was moribund by the time it was examined at necropsy 66 days after infection with 7000 larvae. Phillips and Koltveit (17) concluded from necropsy findings that diarrhea in a naturally infected Shetland pony was associated with immature *S. edentatus* that had caused peritonitis and a proliferative inflammatory reaction of the mesentery of the small colon. However, since this pony also had a "generalized septicemia" with petechial hemorrhages of the myocardium as well as a severe splenitis, it would appear open to question whether *S. edentatus* was the sole or even the primary cause of its clinical signs. The absence of clinical signs noted in our studies may well be due to the fact that ponies are less susceptible to the effects of a horse strain of *S. edentatus*.

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