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بايلز اسكارس بروسيونز (٢) دراسة الشكل الظاهري ومسار العدوي فى الثدييات الصغيرة للطور اليرقى الثالث

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تصيب ديدان البايلز اسكارس بروسيونز حيوان الراكون وتسبب يرقاتها أضرار جسيمة فى الجهاز العصبى والعين والاحشاء الداخلية للانسان والثدييات والطيور • لذلك قام الباحثون لاول مرة بدراسة موفولوجية تفصيلية بواسطة الميكروسكوب الضوئى والميكروسكوب الالكترونى للسطح الخارجى للطور اليرقى الثالث فى مراحله المختلفة ، ومقارنة هذه الدراسة بما سبق دراسته على يرقات الطور اليرقى الثالث لديدان مجموعة الاسكارس الاخري•

كما قام الباحثون بتتبع الاصابة معمليا فى خنزير غينيا حتى الانسلاخ الثانى والوقوف على معرفة التغيرات الباثولوجية واستنتاج أهمية الثدييات الصغيرة كعائل وسيط لاتمام دورة حياة هذا الطفيل •

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BAYLISASCARIS PROCYONIS (STIEFANSKI AD ZARNOWSKI, 1951) ASCARIDIDAE : NEMATODA. II. THIRD STAGE LARVAE, MORPHOGENESIS AND MIGRATORY BEHAVIOUR

(With 19 Figures)

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SUMMARY

Baylisascaris procyonis occurs naturally as a parasite of raccoons (Procyon lotor), and may cause visceral larva migrans and CNS disease in humans, other mammals and birds.

Early third-stage larvae measured 49-60 u in diameter and 928-1059 u in length. Late third-stage larvae measured 75-84 u in diameter and 1479-1676 u in length. The head was rounded and slightly swollen, while the lips were ill-differentiated and not protuberant. The esophagus was clavate, strongyliform, terminated in a pyriform bulb, and constituted about 1/8 of the total length. The lateral alae were well developed. The tail was dorsally curved and hook-like. B. procyonis third-stage larvae were studied by scanning electron microscopy. Three small poorly differentiated lips appeared surrounding the anterior tip of the esophagus. Four primitive button-like flat cell masses were seen on the primary lips corresponding to the external ring of the labial papillae. Three pairs of pulp cells appeared apically on the growing primary lips corresponding to the internal ring of primary labial papillae. Two amphidial pores were located laterally on the primary subventral lips. A dorsal cervical median crest was present on the anterior 1/6 of the body length, flanked by a submedian groove on each side. A dorsal median groove extended along the body to the anal region. The tail was sharply curved dorsally, narrowly and deeply segmented, with a blunt rounded terminal knob.

Somatic migration of the second-stage larvae in occurred experimentally injected guinea pigs, with development of larval granulomaslomas. The second moult occurred within 10-21 DAI. Small mammals as intermediate hosts may be necessary to the life history of B. procyonis.

INTRODUCTION

The genus Baylisascaris (SPRENT, 1968) includes several spocies of ascaridoids, most of wich utilize an intermediate host to complete their life cycle (Sprent, 1953; 1954 and SPRENT et al., 1973). Baylisascaris procyonis (STEFANSKI and ZARNOWSKI, 1951) occurs naturally as a parasite of the raccoon, Procyon lotor. The present paper doscribes the results

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of experimental infection of guinea pigs to establish an animal model intermediate host in order to follow the migratory behavior of the larvae. A detailed description of the third-stage larvae of B. procyonis using light and scanning electron microscopy is reported in order to facilitate reliable differential diagnosis from other nematode larvae in biopsy and necropsy specimens.

MATERIAL and METHODS

Infective eggs of B. procyonis were obtained from the gravid uteri of adult female worms recovered from the intestine of naturally infected raccoons, as previously described (SAKLA et al., submitted). Young (birth-400 gm) and adult (400-650 gm) female Hartley guinea pigs were used for oral infection with 300-5,000 embryonated eggs of B. procyonis. Subcutaneous and intraperitoneal routes of infection with different doses of embryonated eggs also were used. The experimentally infected guinea pigs were observed daily. The animals that did not die naturally from the infection, and had severe CNS signs, were killed at intervals and necropsied. The third-stage larvae were collected from whitish larval granulomas which were seen grossly, distributed on the serous somatic tissues and membranes. Live L3 were killed by moist heat, drawings were made with aid of a camera lucida, and measurements in microns were made using a Zeiss Universal microscope equipped with an ocular micrometer.

Scanning electron microscopy :

Third-stage larvae obtained from the larval granulomas were fixed in 2.5% glutaraldehyde - 4 mM $CaCl_2$ - 64 mM Na cacodylate buffer containing 131 mM sucrose. Larvae were dehydrated in ascending concentrations of ethanol, washed three times in amyl acetate, placed on a glass coverslip, dried at room temperature over silica gel, sputter coated with 100 A gold, and examined with an AMRAY 1400 scanning electron microscope.

RESULTS

Morphology of the third-stage larvae by light microscopy :

Most of the third-stage larvae had developed through the second moult by 10-21 days after infection. The first change noted in the growing fthird-stage larvae was an increase in the total length and width. Third stage-larvae are more slender than the second stage larvae, with a rounded, slightly swollen, anterior end, and their anatomical details are more easily discerned. Early third-stage larvae measure 928-1059 u in length and 49-61u width, while the late third-stage larvae measure 1479-1676 u in length and 75-84 u in width. The body cuticle appears beaded due to prominent transverse striations or annulations on the cuticle of the larvae proper, from the base of the lips to the tail. The lateral alae are single, well developed and extend all along the body commencing halfway between the anterior end and the nerve ring. The lips are small, ill-differentiated, and not constricted, nor do they appear to protrude from the body proper (Figs. 1-4). The labial cuticle appears granular, indented and surrounds the esophageal tip externo-laterally. The esophagus is a clavate, srongyliform type and has a uniform diameter tube terminating in a pyriform bulb. It measures from 189 to 232 u in length and measures about 1/8 of the total length of the late third-stage larvae. The nucleus of the dorsal oesophageal gland can be distinguished. The nerve ring appears as a clear strongly fibrillar structure of the mid-esophageal region. The two excretory columns are developed, the excretory cell nucleus shows changes in structure and size relative to the second-stage larva (SAKLA et al. submitted, 1988), and the excretory pore is found on the ventral surface 110-115 u from the anterior end (Figs. 1, 2).

I. Migratory Phase :

The second-stage larvae arrived in lung and liver within 3-5 days after infection (DAI). Clinical signs commenced on the second DAI, including fever and a disinclination to move or feed, and were followed by pulmonary signs in the form of dyspnoea which became very evident on 3 DAI. In heavily infected animals (3,000 eggs), the guinea pigs usually died on the 6th day after infection. The lesions observed at necropsy in the guinea pigs at this stage were severe congestion of the visceral organs particulary the intestinal wall and mesenteric tissue. Red patches, spots and streaks appeared on the lungs and liver, and there as hepatomegaly. In guinea pigs given lower doses (500-1,500 eggs), it was observed that the number of the second stage larvae rapidly decreased in the lungs and liver ater the 6-8 DAI. Signs of weakness, emaciation and hypoxia were evident folowed by diarrhea or alopecia in some animals. The lesions of the lungs became patches of dark red hepatization and those of the liver were pale in color with white spots.

II. Encapsulation and moulting phase :

The second-stage larvae started to migrate directly out from the thoracic and abdominal viscera by 9 DAI, becoming dispersed into different somatic tissues and encapsulated inside white nodules. The majority of the white nodules were distributed on the pleural, pericardiac and omental serous membranes (Fig. 16). Larval granulomas were seen also in small numbers on the surface of the lung, the thoracic surface of the diaphragm, the pulmonary surface of the intercostal muscles, in the superficial layer of the myocardium, and in the serosa of the small intestine and oesophagus. The encapsulated second stage larvae commened to undergo their second moult within 10-21 DAI inside the larval granulomas.

Guniea pigs which received from 500 to 1,500 embryonated eggs usually died within 14 to 21 days after infection. The most common autopsy lesion observed in these animals was fatty degeneration of the liver. Guinea pigs infected with 300-400 embryonated eggs survived up to 18-22 days, and died with severe nervous symptoms thereafter. Signs of CNS dysfunction consisted of locomotor incoordination, circumambulation, head tilt and tremors. Ptosis of the eye lids, probably due to dysfunction of the cranial nerves, were observed in some animals.

Guinea pigs became semicomatose and recumbent and died within two days of the onset of the CNS signs. The autopsy lesions observed in animals dying with CNS signs were generalized encephalitis, congestion and hemorrhages in the cerebrum and cerebellum. Histologically, migration tracks and a few third-stage larvae were seen in the CNS tissue, accompanied by perivascular infiltration with eosinophils and lymphocytes.

Structure of the larval granu lomas :

The spherical or oval macroscopic whitish nodules, 1-2 mm in diameter, contained one or rarely two living third-stage larvae inside. The viable larvae were surrounded by a pool of inflammatory cells, composed principally of eosinophils and lymphocytes, and were located eccentrically in the granulomas (Figs. 17-19). The cast off skin of the second larval moult, surrounded by neutrophils, macrophages and plasma cells, occupied the rest of each granuloma. The granuloma was peripherally surrounded by fibroblasts, epithelioid cells and a well circumscribed coallagenous capsule. Pleural and mesentric larval granulomas were observed to be attached to the serous membranes by a stalk. Clusters of larval granulomas also were seen in the mesenteric tissues. Larval granulomas of this type were not noted in the livers, eyes or brains of the infected animals.

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The esphagointestinal valve is triangular in shape, and the intestine appears as a tube with an open lumen lined by single layer of long rectangular cells. The rectum opens to the anal orifice which is located about 72-75 u from the end of the tail. The slender fusiform body tapers abrouptly to a characteristic dorsally curved hook-like tail (Figs. 1,5). The genital primordium appears as an ellipsoidal mass of cells located ventrally in the mid-posterior region of the intestine. Rectal caudal glands are distinguished in the third-stage larvae (Fig. 1).

Scanning electron microscopy of the third-stage larvaes :

The anterior end of the third-stage larvae is round and slightly swollen (Figs. 6-12). In the enface view, a triradiate Y-shaped stoma laeds directly into the esophagus (Fig. 12). The most anterior tip of the esophagus, consisting of one dorsal and two subventral thick fleshy protrusions, are more obviously seen in the early third-stage larvae (Fig. 10). In the fully developed third-stage larvae, three small poorly differentiated lips appear surrounding the anterior tip of the oesophagus. Three pairs of pulp cells terminating in a minute granule are visible apically on the growing primary lips surrounding the anterior tip of the oesophagus, representing the internal ring of the primary labial papillae. The external ring of the primary labial papillae is represented by four primitive button-like flat cell masses, two dorsolateral on the dorsal primary lips, and one ventrolateral on each subventral primary lip, clearly visible in the early third-stage larvae (Fig. 11). In the fully developed third-stage larvae, the three primary lips were partially separated by a marked interlabial notch. Two shallow grooves behind the notch between the primary dorsal and subventral lips were easily seen. Three interlabial ridges or bands between the primary lips were readily distinguished, and appear where the anterior edges of the cephalic tissues meet the somatic tissues of the esophagus (Fig. 12).

Two amphidial poros are located laterally and slightly dorsally on the basal portion of each primary subventral lip (Fig. 13). The amphidial pores open into an amphidial channel which can be followed laterally through the amphidial ridge. The dorsal cuticular morphology of the anterior end of the third-stage larvae differs from the rest of the body. A dorsal cervical median crest or ridge can be seen, commencing from the anterior extremity and ending behind the esophageal bulb, extending about 1/6 of the body length from the anterior end (Fig. 6). A submedian deep groove is present on each side of this dorsal cervical crest. The trilobed appearance of the dorsal surface at the oesophageal level is evident in transverse sections. A dorsal median groove extends all the way along the body commencing behind the end of esophagus to the level of the anal region. Cross sections of the larvae behind the oesophageal region at the intestinal level are bilobed dorsally. Lateral alae are prominent along the sides of the body in the lateral field commencing anteriorly from the less prominent amphidial ridges, and extending all the way along the body to the tail. The ventral cuticular morphology of the larvae is distinctly striated and nearly flat (Fig. 7).

The tail in this stage s sharply curved dorsally, and is narrowly and deeply segmented with a blunt rounded terminal knob, giving the tail a resemblance to that of a rattle snake (Fig. 14, 15). The anal orifice is situated in the mid-ventral line posteriorly and is surrounded by an elevated rectangular cuticular thickening (Fig. 14).

Migratory behavior :

In order to investigate the migratory route of B. procyonis larvae and their distribution and development in the various parts of the intermediate host, 30 guinea pigs were infected with 300 to 5,000 embryonated eggs. The experimentally infected guineapigs showed the presence of two different phases of larval development.

DISCUSSION

B. procyonis third-stage larvae obtained from experimentally infected guinea pigs were easily distinguished from the second-stage larvae by both light and scanning electron microscopy. Differences were found in the length and equatorial width. The clavate strongyliform esophagus was a reliable criterion for distinguishing third-stage larvae of B. procyonis from second stage larvae, as described for other nematodes (LEVINE, 1980). Furthermore, the distinctive appearance of the head is evidence of differentiation from the second stage. The development of the functional primary labia, and the appearance of the primary labial papillae, were characteristic of the third-stage larvae. This agrees with the findings of ROBERTS (1934) for Ascaria lumbricoides and DOUVRES et al. (1969) for A. suum. An additional distinguishing feature is the dorsally curved hook-like tail which was evident using both light and scanning electron microscopy.

The literature does not contain SEM micrographs of Baylisascaris third-stage larvae, except for B. tasmaniensis described by SPRENT et al. (1973). OUr findings for the labial organization of B. procyonis third-stage larvae provide useful comparative characteristics for this group. The appearance of four button-like flat cell masses, two on the dorsal primary lip and one on each of the primary subventral lips, in the early third-stage larvae, corresponded to the four large double papillae of the external ring of labial papillae of the adult worms described by KAZACOS and TUREK (1982). The development of the three pairs of pulp cells on the anterior tip of the primary lips surrounding the oesophagus corresponded to the internal ring of labial papillae. KAZACOS and TUREK (1982) described the internal ring of papillae of B. procyonis adult worms as two distinct pits in the smooth apical portion of each lip, at apprimately 11 O'clock and 1 O'clock.

The growing primitive lips of the third-stage larvae were found to be less differentiated than the labial organization of the adult worms described by KAZACOS and TUREK (1982). As growth proceeded, the developing lips appeared to move anteriorly to cover the protuberant tip of the oesophagus. The location of the esophageal opening at the base of the three lips in adult B. procyonis (KAZACOS and TUREK, 1982) supports the hypothesis that the labia arise during development from a forward displacement of cuticle around the tip of the esophagus. The location of the amphidial pores inthe posterior dorsal quadrant of the subventral lips of the adult worm (KAZACOS and TUREK, 1982) provides a further marker for this region of cuticle during development. In the L2, these are located posterior to the lip-like esophageal protuberances (SAKLA et al., Submitted). In the L3, the amphidial pores are located more anteriorly, on the cuticular collar which develops around the tip of the esophagus.

The junction of the primary lips and the body proper in the late third-stage larvae was marked by a slight constriction posterior to a rounded head. This junction may differentiate into a postlabial groove in the adult worms, as described for B. tasmaniensis by SPRENT et al. (1973). The interlabial notch observed in the late third-stage larvae may correspond to a distinct interlabial space described by SPRENT (1973) for B. tasmaniensis adult worms. A corresponding interlabial space is seen in SEM's of B. procyonis adults (KAZACOS and TUREK, 1982).

The morphology of the cuticular surface and of the tail provide useful featurers for the identification of B. procyonis larvae in tissue sections. The dorsal surface at the level of the esophagus and at the midgut shows a cuticular ridge flanked by two grooves, evident both by SEM and in sections. The lateral alae are thick, fleshy and flanked by cuticular ridges. The sharply curved, knobbed tail is unique among ascarids (DOUVRES et al., 1969).

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B. procyonis has significant pathogenic capabilities, marked contaminative ability and aggressive larval migratory behaviour (KAZACOS et al., 1984). So far, fatalities in two young children in the United States were attributed to B. procyonis (HUFF et al., 1984 and FOX et al., 1985). In our studies guinea pigs were used as an animal model intermediate host for B. procyonis. Experimental infections of mice, hamsters, gray squirrels, woodchucks and subhuman primates were studied by KAZACOS et al. (1985). KAZACOS et al. (1985) used oral doses of eggs rangingfrom 3 to 33 eggs per gram body weight and observed dose-dependent ocular invasion by migrating larvae ranging from 9% to 25% of animals infected. Doses of 6 eggs/gm were uniformly fatal to guinea pigs within 2-4 days in our studies. At doses from 0.25-2 eggs/gm, rapid death occurred in our studies from CNS disease or fatty degeneration of the liver within 14-21 days. We did not observe ocular penetration by larvae after feeding of eggs, possibly because the lethal dose for guinea pigs is below that which produced significant ocular invasion in the other species studied by KAZACOS et al. (1985). The ability of B. procyonis larvae to invade the guinea pig eye was established by subconjunctival injection of newly hatched L2 (unpublished observations).

Data of the larval migratory behaviour obtained in the present study suggest that the portal blood system may be the common migratory pathway for the second-stage larval invasion of the liver and lung of the intermediate host during the first few days after infection. However, it is also possible that a few larvae migrated directly out from the intestinal wall into the peritoneal cavity and retroperitoneal tissues. The somatic migration of the second-stage larvae, thedevelopment of the larval granulomas, the occurence of the second moult within 10-21 DAI, the growth of third-stage to a length of 1-1.7 mm, and the encapsulation of L3 in the tissues for an indefinite period may occur in mammals naturally infected with B. procyonis. It appears that this parasite in the natural environment could involve small mammals as intermediate hosts which are then eaten by raccoons. Further investigations by infecting raccoons with infective eggs of B. procyonis, to determine whether the intermediate host is obligatory or not, are necessary. Of the previous investigators, TINER (1949) reported that skunks could be inected with A. columnaris by feeding them mice harbouring the encapsulated larvae of this parasite. SPRENT (1953) concluded that the life history of A. devosi requires a true intermediate host for its completion. SPRENT (1973) used laboratory mice as an experimental intermediate host to infect Tasmanian devils with B. tasmaniensis.

The large, rapidly growing and highly invasive larval stages of B. procyonis are capable of producing serious ocular and CNS disease in humans (FOX et al., 1985 and KAZACOS et al., 1985). Identification of these larvae in tissue sections may be made on the basis of size. Our study of the morphogenesis ofthe third stage larvae has revealed additional morphological features which may be used for conclusive identification of B. procyonis.

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FIGURE LEGENDS

Fig. (1): Camera lucida drawing, B. procyonia third stage larva, lateral view. A, anterior end; B, posterior end; A, anus; DESN, nucleus of dorsal esophageal gland; EB, esophageal bulb; EC, excretory column; EN, nucleus of excretory cell; EP, excretory pore; ET, excretory tubule; ES, esophagus; EV, esophago-intestinal valve; GN, ganglionic nucleus; INT, intestine; NR, nerve ring; RGN, rectal gland nucleus.

Fig. (2): B. procyonis third stage larva. Differential interference phase contrast.

Fig. (3): B. procyonis third stage larva, Anterior end.

Fig. (4): B. procyonis third larva, haematoxylin stain, anterior end.

Fig. (5): B. procyonis third stage larva, posterior end.

Fig. (6): SEM of B. procyonis third stage larva, showing the dorsal aspect of the anterior end (Bar, 100 um).

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Fig. (7): B. procyonis third stage larva, showing ventral view of the anterior end (Bar, 100um).

Fig. (8): B. procyonis third stage larva, lateral view of the anterior end (Bar, 10 um).

Fig. (9): B. procyonis third stage larva, dorsal view, showing dorsal grooves and lateral alae (Bar, 10 um).

Fig. (10): B. procyonis early third stage larva, anterior end. Note infolding between the amphidial pore and the tip of the esophagus, and the primitive latero-dorsal and ventral labial papillae (Bar, 10 um).

Fig. (11): B. procyonis early third stage larva, anterior end, A later stage of development than Figure 14, the head appears more rounded and the developing labial papillae appear as discrete button-shaped masses (Bar, 10 um).

Fig. (12): B. procyonis late third stge larva, showing the rounded swollen head. A shallow groove is visible between the developing dorsal and subventral lips and interlabial ridges have appeared between the developing lips and the tip of the esophagus (Bar, 10 um).

Fig. (13): B. procyonis late third stage larva, anterior end, lateral view (Bar, 10 um).

Fig. (14): B. procyonis third stage larva, posterior end. A raised, oval cuticular bleb is present anterior to the anus, and the tail is sharply curved dorsally (Bar, 10 um).

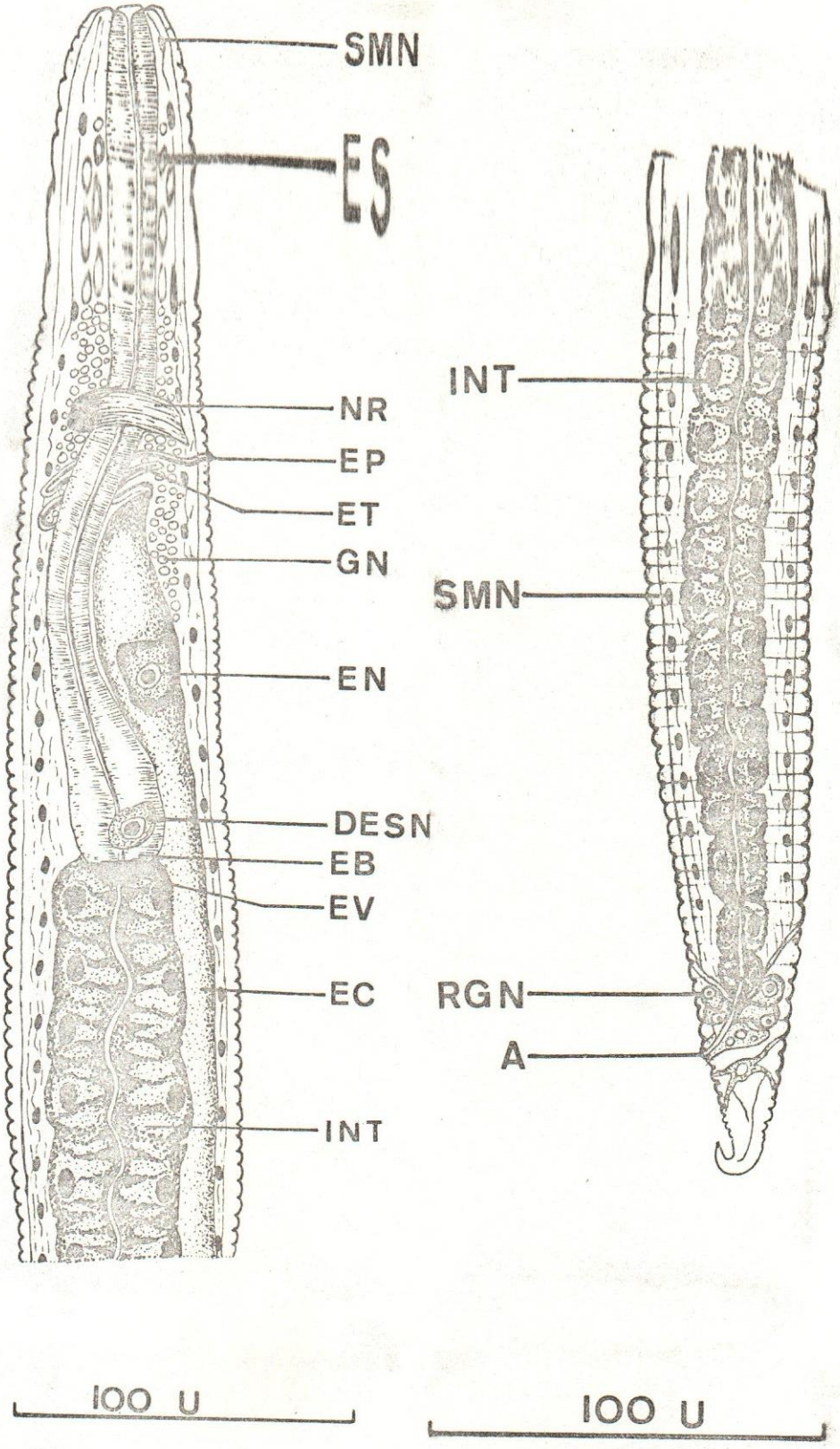
Fig. (15): B. procyonis third stage larva, posterior end. The dorsally curved tail terminates in a rounded knob (Bar, 10 um).

Fig. (16): Thoracic surface of diaphragm of an experimentally infected guinea pig 10 days after infection. Multiple spherical larval granulomas are present on the surface.

Fig. (17): Larval granuloma on the thoracic surface of the diaphragm in an experimentally infected guinea pig (Haematoxylin and eosin).

Fig. (18): Larval granuloma on pleural membrane of an experimentally infected guinea pig (Haematoxylin and eosin).

Fig. (19): Larval granuloma on the surface of the lung of an experimentally infected guinea pig (Haematoxylin and eosin).



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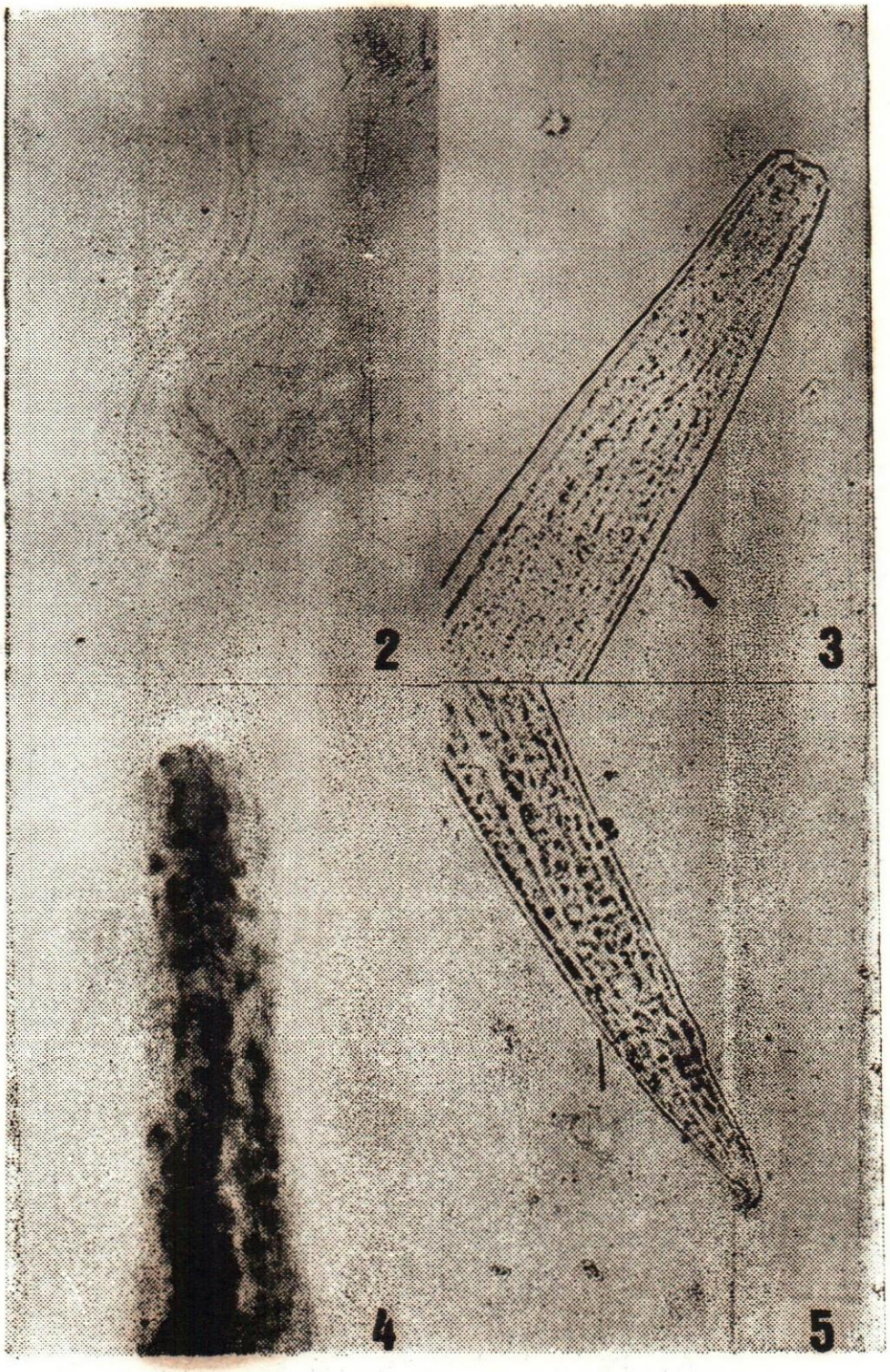
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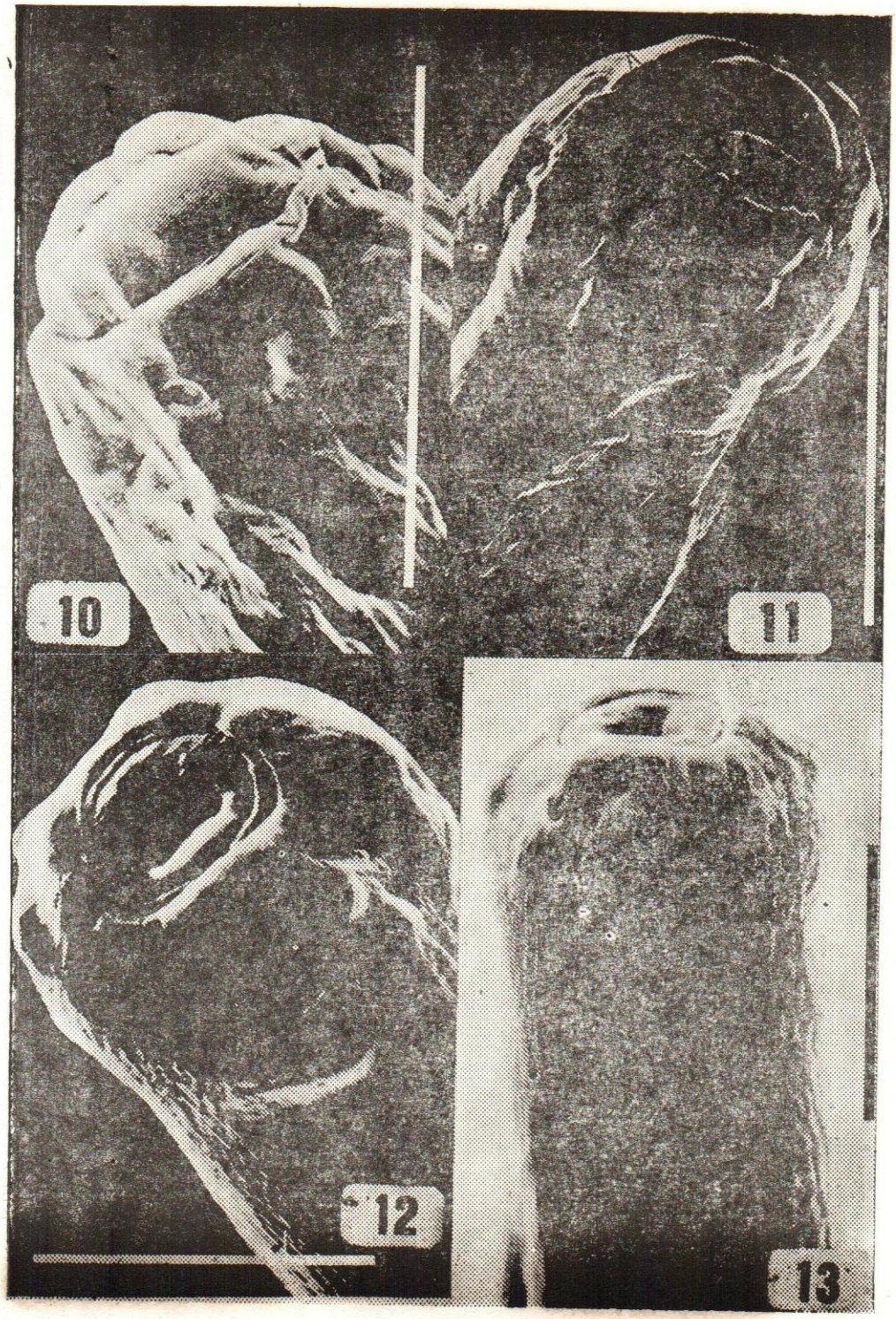
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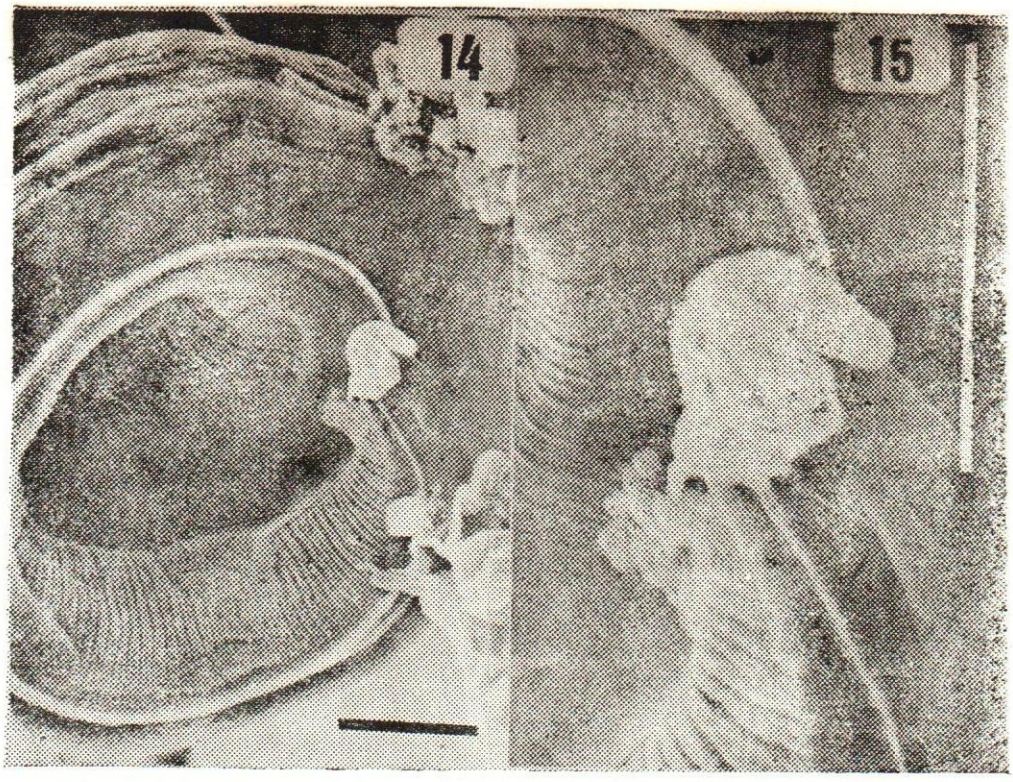
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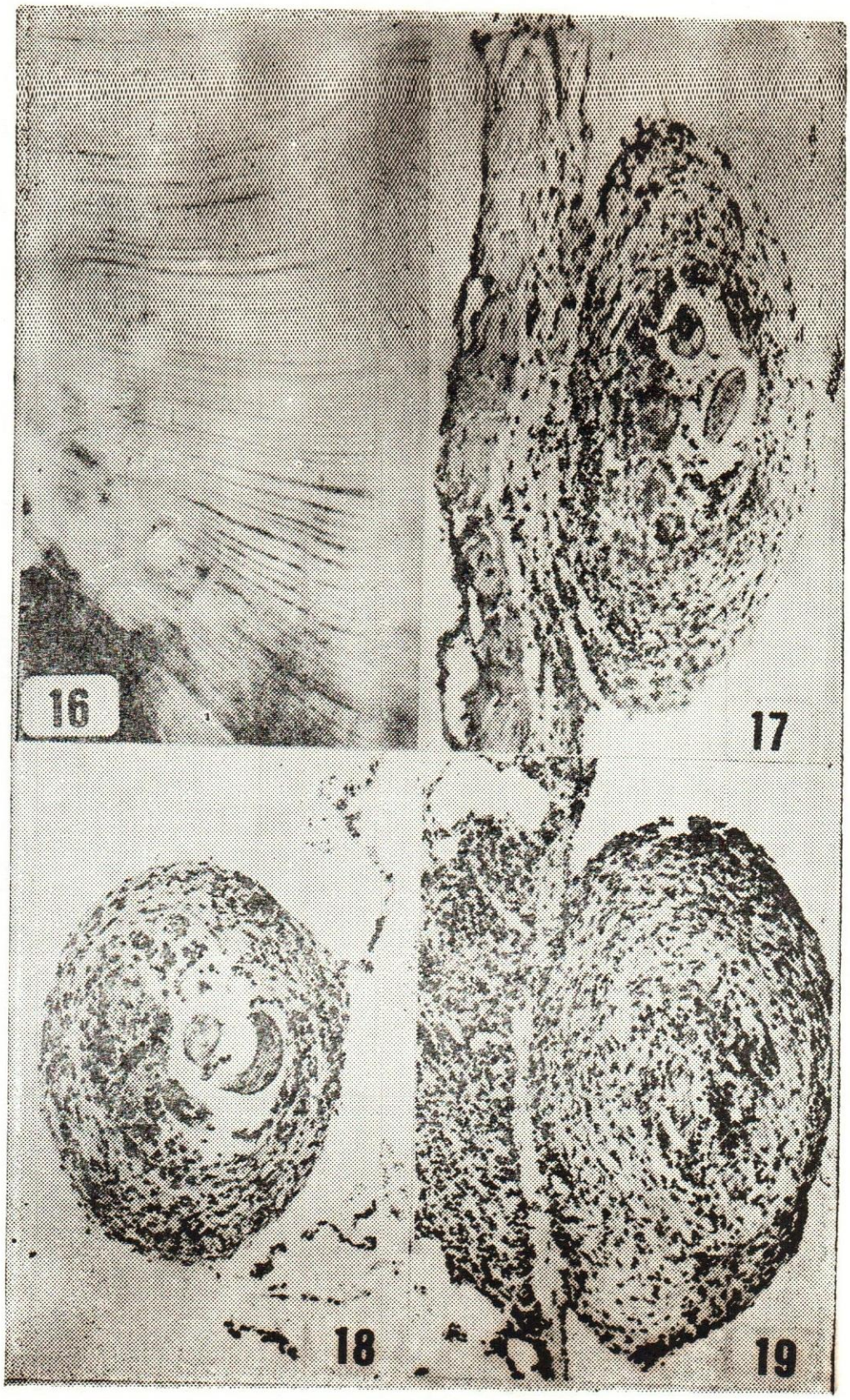
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