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EXPERIMENTAL HETEROXENOUS CYCLE OF *LAGOCHILASCARIS MINOR* LEIPER, 1909 (NEMATODA: ASCARIDIDAE) IN WHITE MICE AND IN CATS

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Reports of natural infections of sylvatic carnivores by adult worms of species similar to Lagochilascaris minor *in the Neotropical region led to attempts to establish experimental cycles in laboratory mice and in cats. Also, larval development was seen in the skeletal muscle of an agouti (*Dasyprocta leporina*) infected* per os *with incubated eggs of the parasite obtained from a human case.*

In cats, adult worms develop and fertile eggs are expelled in the feces; in mice, larval stages of the parasite develop, and are encapsulate in the skeletal muscle, and in the adipose and subcutaneous connective tissue. From our observations, we conclude that the larva infective for the mouse is the early 3rd stage, while for the final host the infective form is the later 3rd stage. A single moult was seen in the mouse, giving rise to a small population of 4th stage larvae, long after the initial infection.

Key words: *Lagochilascaris minor* – experimental cycle – final and intermediate hosts

Of the ascarids that infect man, *Lagochilascaris minor* Leiper, 1909 develops in the human host all forms in the life cycle: eggs, larvae, and fertile adults (Oostburg & Varma, 1968; Oostburg, 1971), giving rise to an unusual clinical picture of morbidity, due to the frequency in which it attacks the cephalic region (Fraiha Neto et al., 1989), producing tumors in the soft tissues and infecting those cavities open to the air. The mechanism of infection remained unknown.

The infection has also been reported in the cat (Fraiha Neto et al., 1984) and in the dog (Sturion et al., 1982; Vidotto et al., 1982), these animals showing tumors and fistulas in the upper repiratory tract, lesions containing adults, larvae, and eggs of the parasite. Sylvatic carnivores have also shown infections with similar ascarids; an ocelot of Costa Rica, *Felis pardalis mearnsi* had worms in the larynx (Brenes-Madrigal et al., 1972). Also, a wild dog, *Speothos venaticus,* was found with parasites in the upper respiratory tract. As with the feline, there were no tumors or fistulas, only the adult worms; there were abundant eggs in the feces (Volcán & Medrano, 1991).

Recently, successful experimental infections have been made in rodents, by oral administration of eggs from a human host of *L. minor.* Larval stages develop preferentially in the skeletal muscle and connective-adipose tissue. However, development in rodents differs for different families. In the agouti *Dasyprocta leporina* (Dasyproctidae) 3rd-stage larvae were encysted in the muscle, with little adjacent tissue reaction and without intracapsular abscesses (Volcán & Medrano, 1990). In the mouse (Muridae), there were abscesses within the capsules and local inflammation. Campos & Freire (1989) reported adult forms in the parasitized nodules of this host.

The present work reports results of experimental infections and proposes a heteroxenous natural sylvatic cycle for *L. minor.*

MATERIALS AND METHODS

Infective material – Eggs were obtained from the feces of a human case of *L. minor,* colonized by the parasite in the paranasal si-

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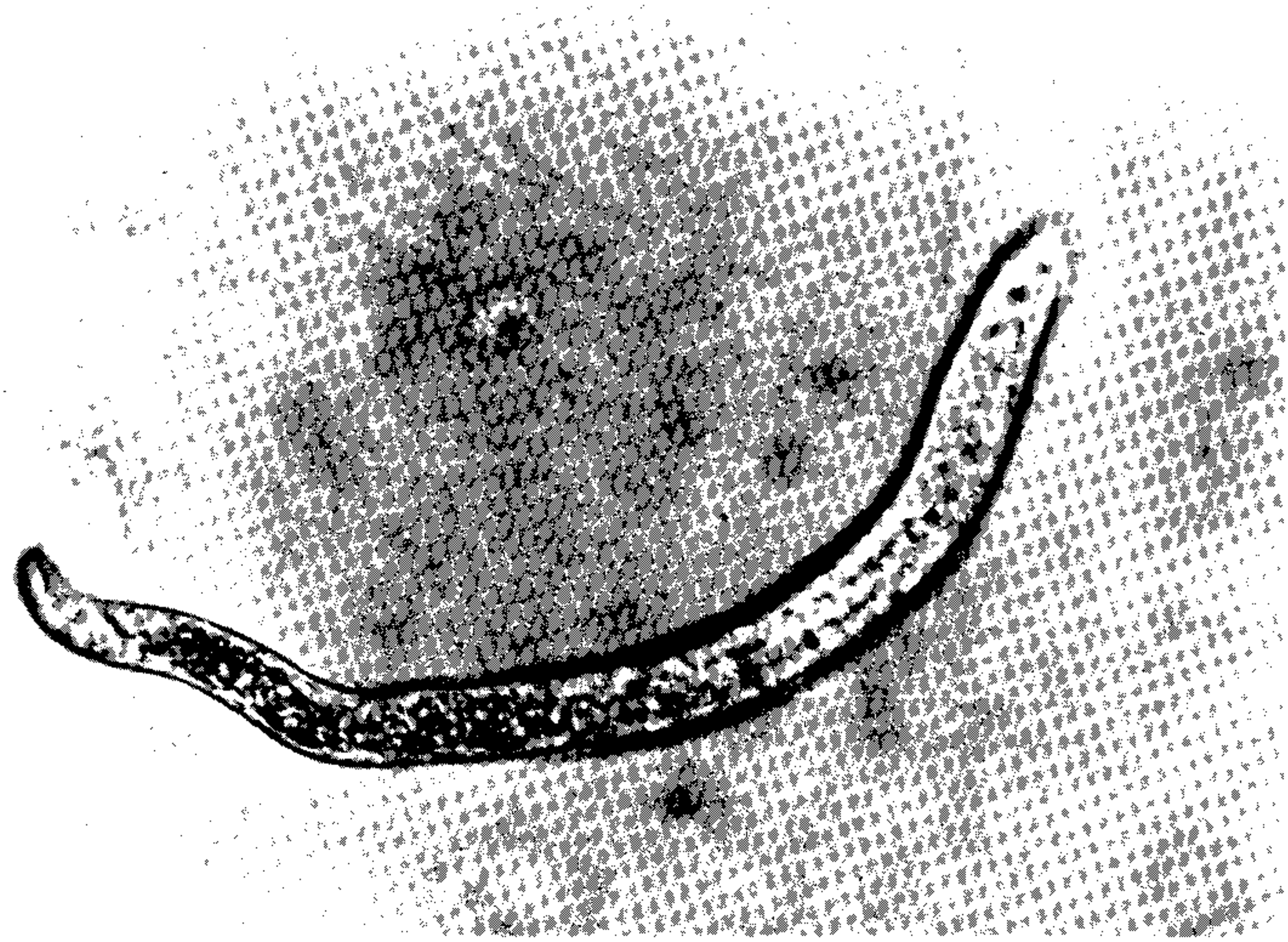

Fig. 1: larva hatched 3 h PI, in the intestinal content of a mouse infected with eggs of *Lagochilascaris minor*. The enveloping sheath can be seen. 500 x.

Fig. 2: histological section of mouse liver 6 h PI. Section of a larva lacking a sheath in the lumen of the central vein of a hepatic lobule. HE, 500 x.

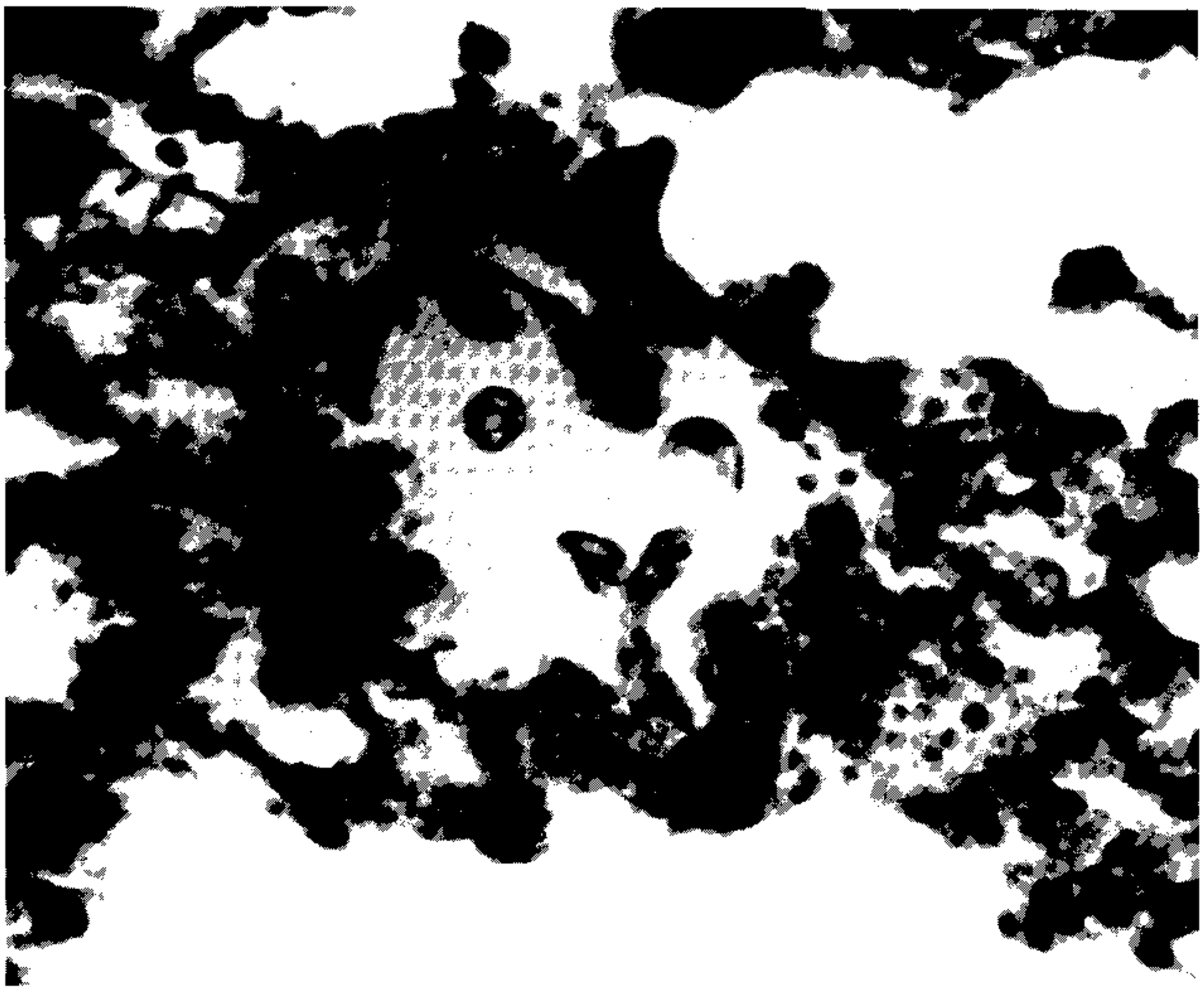

Fig. 3: histological section of mouse lung, 3 days PI. In the lumen of an alveolar duct, surrounded by folded alveoli, various sections of a larva of *Lagochilascaris minor* can be seen. HE, 500 x.

nuses (Volcán et al., 1982). Eggs were incubated in 1% potassium bichromate ($K_2Cr_2O_7$) for more than 30 days. Prior to inoculation, eggs were treated for 2 min with 5.25% sodium hypochlorite (NaOCl), and then washed 6 times with distilled water.

Infections of mice – Adult Swiss white mice from the animal facility of the School of Medicine of the Universidad de Oriente, of both sexes, and weighing 30-45 g, were inoculated orally with 300-600 eggs per animal. Of a total of 70 mice infected, groups of 2-4 mice each were sacrificed and autopsied at the following intervals post-inoculation (PI):

First day: 1, 2, 6, 8, and 12 hours
First week: daily
First 2 months: 10, 15, 20, 30, 40, 50 days
First 6 months: monthly, and afterward bimonthly for a full year.

At each necropsy, the digestive tract, heart-lung block, and skeletal muscle were examined to detect nodules. When these became visible in the muscle or adipose tissue, they were extracted and the larvae removed for identification. Also, samples were reserved for making stained histological sections.

Infections of cats – Three groups of cats (*Felis catus domesticus*), obtained from the local population, of more than 3 months age, and of no specified breed, were inoculated. Their feces were examined to preclude the presence of eggs of other ascarids. The first group, 10 animals of both sexes, were allowed to feed on 1 or 2 mice proven to have abundant parasitic nodules, at more than 40 days after inoculation with *L. minor*. The second group of 5 cats were given mice inoculated 3 days previously, which had shown larvae in the lungs. The third group of 3 cats were inoculated with some 5000 eggs by the oral route.

The feces of cats were examined daily after inoculation to detect eggs of parasite. When these appeared, the cats were sacrificed some days or weeks later, and the viscera and muscles were carefully examined, especially the upper respiratory tract. Cats showing no eggs in the feces were sacrificed 3 to 6 months post-inoculation.

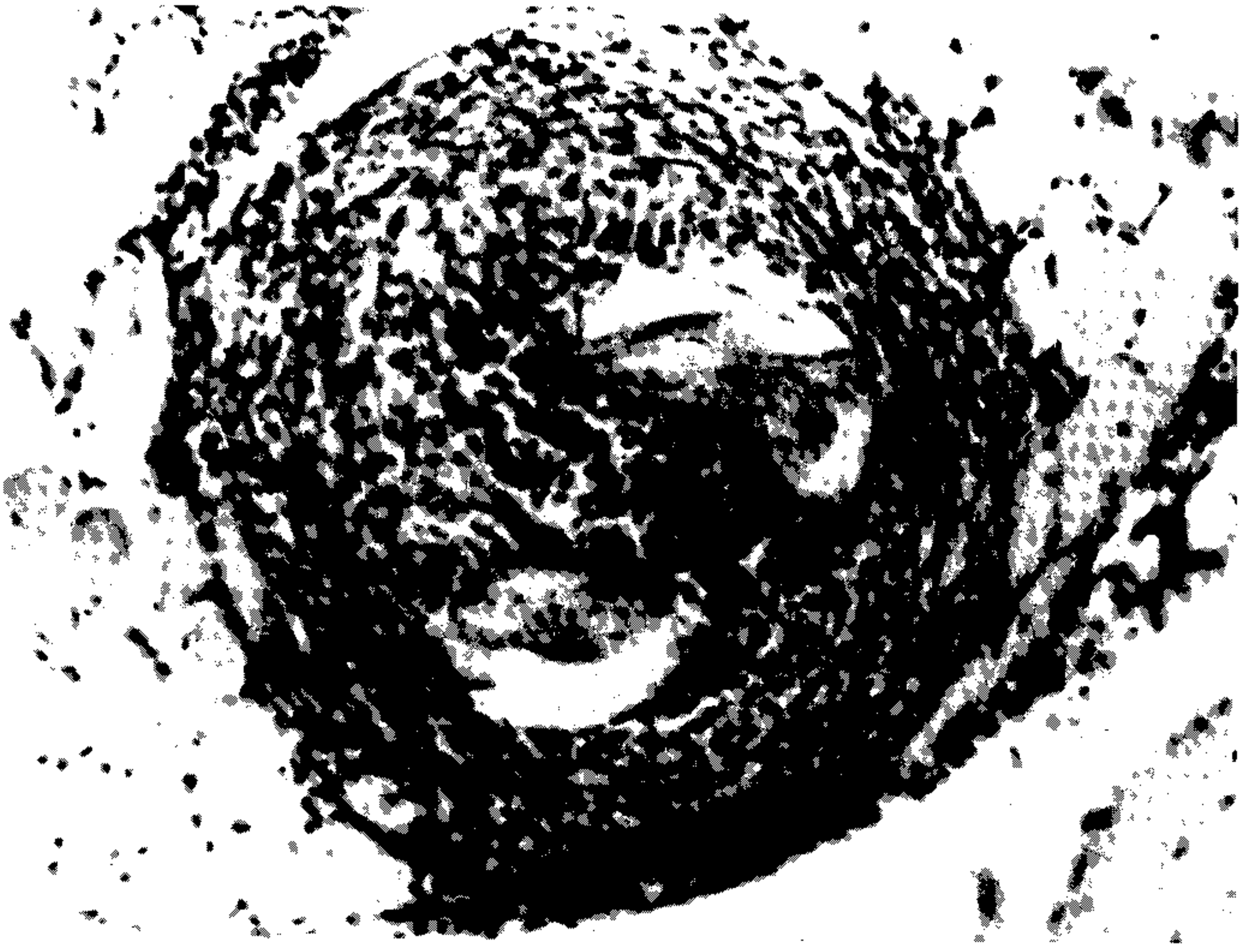

Fig. 4: section of skeletal muscle of mouse 20 days PI, showing a nodule of connective tissue containing a section of a larva of *Lagochilascaris minor* and material from its digestion. HE, 200 x.

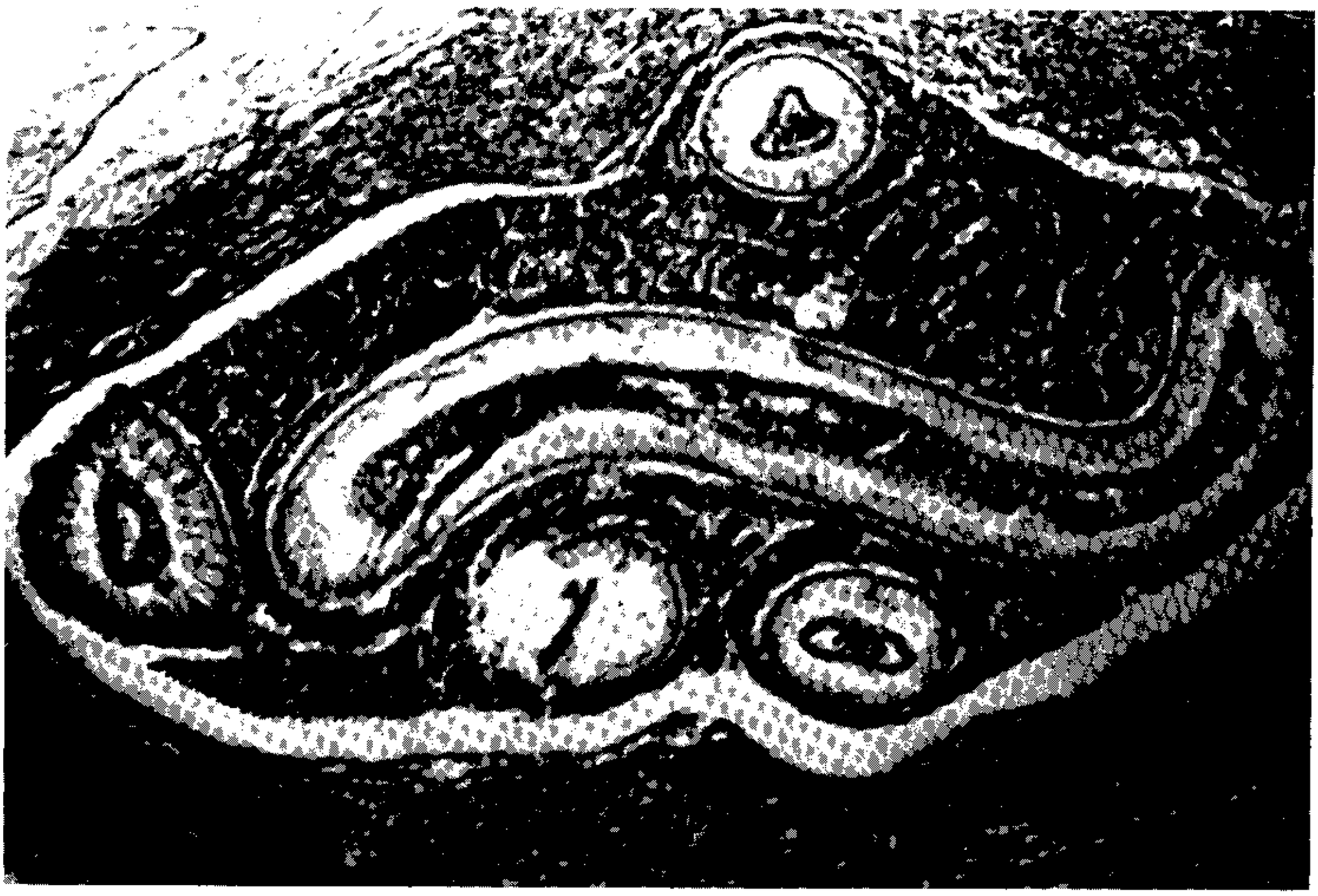

Fig. 5: section of a fibrous nodule from muscle of mouse 60 days PI. The material excreted by a larva of *Lagochilascaris minor* is organized around the parasite. HE, 78 x.

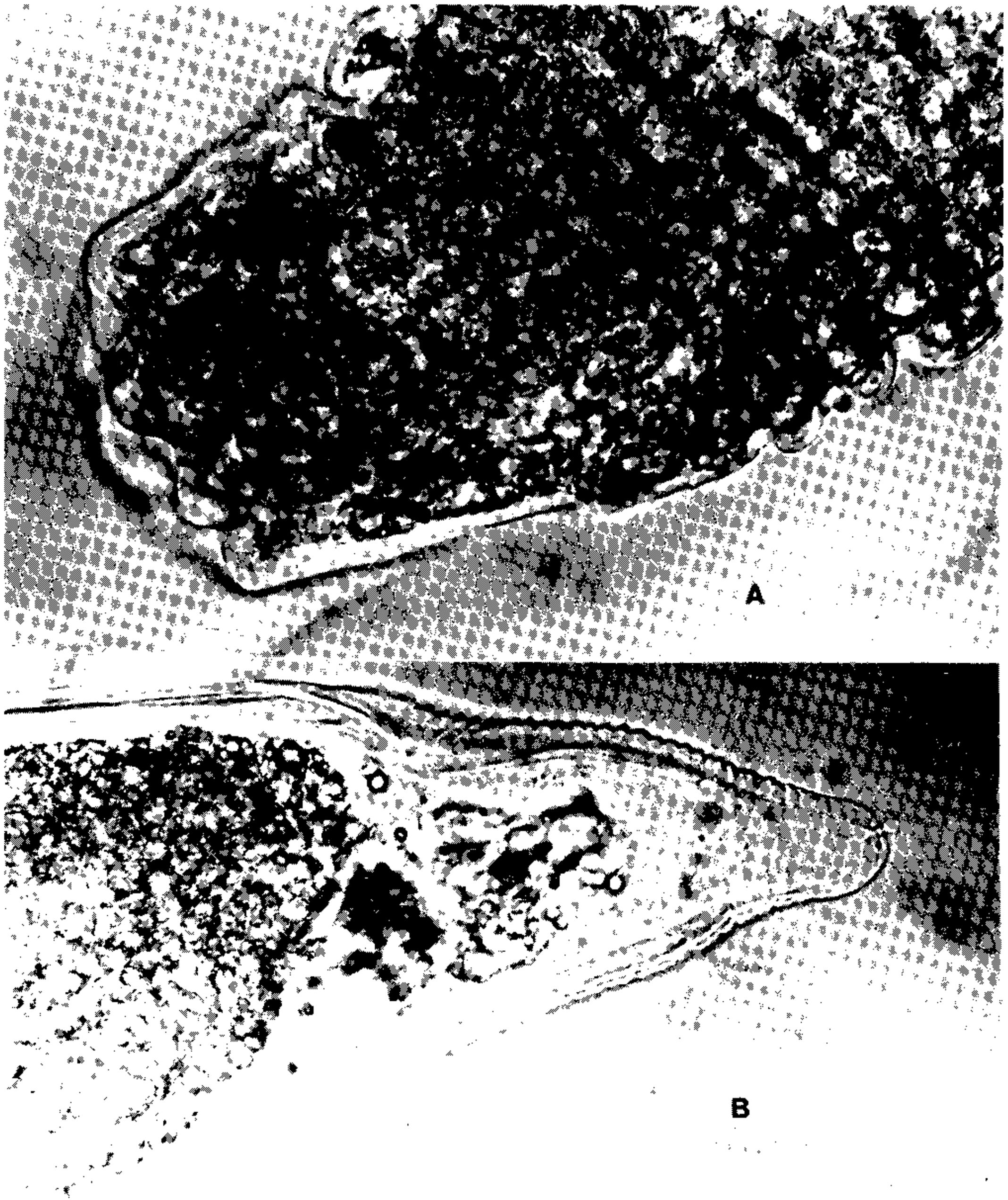

Fig. 6: A – Anterior extremity of the third molt of *Lagochilascaris minor,* in the skeletal muscle of a mouse 220 days PI. The form of the molted sheath preserves the shape of the labia of a third-stage larva, and the post-cephalic groove is absent. Mounted in alcohol/ glycerol, 500 x. B – Posterior extremity of the above. The sheath shows the filiform shape, with the terminal globosity, characteristic of the third-stage larva.

The early stages of parasites, obtained at necropsy, were preserved in 5%/70% glycerine/alcoohol; the advanced larval stages and the adults were treated with hot glacial acetic acid, washed, and preserved in glycerine/ alcoohol, as above.

RESULTS

Similar to what is seen when larva-containing eggs of *L. minor* are compressed between slide and coverslip, the larvae which have hatched naturally in the intestine of the mouse some 3 h post-inoculation are protected by 1 or 2 residual sheaths, representing the intraovular moults of the parasite. The sheaths have the same shape as the distal portion of the larva in their posterior part – a filiform prolongation surrounded by a spherical thickening (Fig. 1). At 6 h PI, larvae are encountered in transit through the liver, following the central vein of the lobule; these larvae lack the

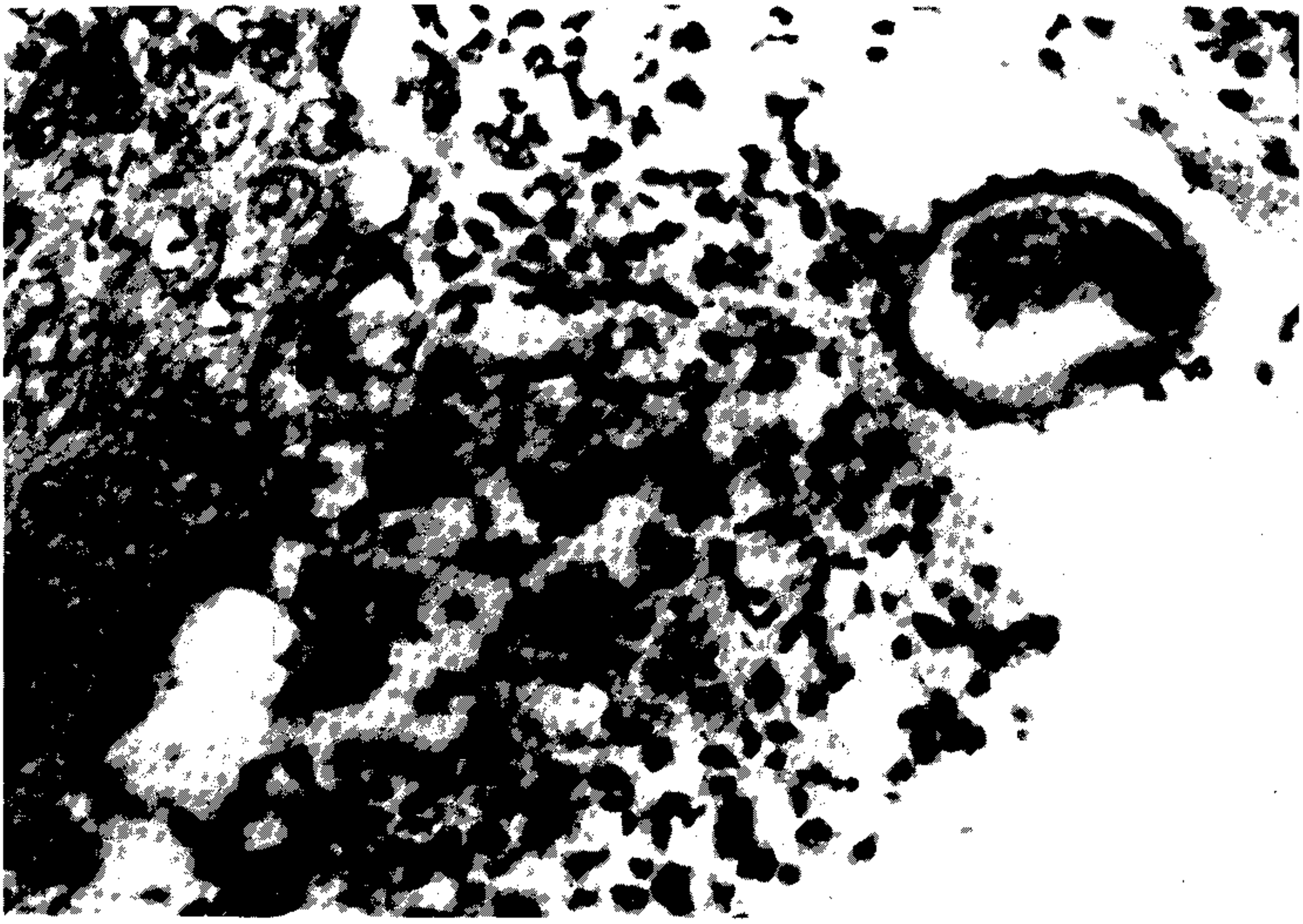

Fig. 7: histological section at the level of the opening of the pharynx, from a domestic cat infected with *Lagochilascaris minor,* 60 days PI. At one edge of the fild, islets of stratified epithelium; at the center, necrotic tissue; an in a cleft, the embryonated egg of *L. minor.* HE, 500 x.

sheath (Fig. 2). At 3 days PI, larvae are seen the bronchioles and alveoli of the lungs, these forms being lightly longer and having larger and more numerous nuclei (Fig. 3). At 5 days PI, the larvae have begun to anchor in the muscle and adipose tissue, where they begin to encapsulate. At 10 days PI, the nodules are not yet visible, but at 14 days, small nodules, occupied by larvae of some 1.2 mm in length can be seen. The reactive tissue englobing the nodules has a concentric lamellar structure with lymphocytic infiltration of mononuclears and eosinophils. This larva has a morphology compatible with an early 3rd stage, similar to what is seen at 20 days PI, when the larvae have reached 2.4 mm in length (Fig. 4).

In later weeks, nodules can be found in tissues of the mouse, empty or containing a larva. At 40 days PI, the larvae are in the advanced 3rd stage, varying in length from 4.4 mm to 7.6 mm; this may indicate sexual dimorphism. At 60 days PI, some of the mice contain advanced 3rd stage larvae, while others have early 4th stage, these latter being easily recognizable by the labia similar to those of the adult, though somewhat flattened, by a definite post-cephalic furrow, and by the immature sexual organs (Fig. 5). Development of the larvae in the infected mice is extremely variable; at 150 days PI, larvae can be found in partially caseous nodules; at 220 days, though most larvae are still of the advanced 3rd stage, there can be found larvae still ensheathed, 4th stage larvae lacking the sheath, and nodules containing only a moulted sheath. The sheaths had a thick cuticle, with the posterior portion as a filiform prolongation having a spherical thickening at the end (Fig. 6).

All mice challenged with inoculations of eggs were positive for the infection.

Ninety percent of the cats infected by consuming mice having infections of more than 40 days PI were positive. Most showed signs of infection some 9 days PI, these signs being a slight decrease in motor activity, weak vocalizations, and frequent sneezing. Some 17 to 20 days PI, eggs of *L. minor* appeared in the feces, and on sacrificing the animals, adult worms were found on the surface of the lar-

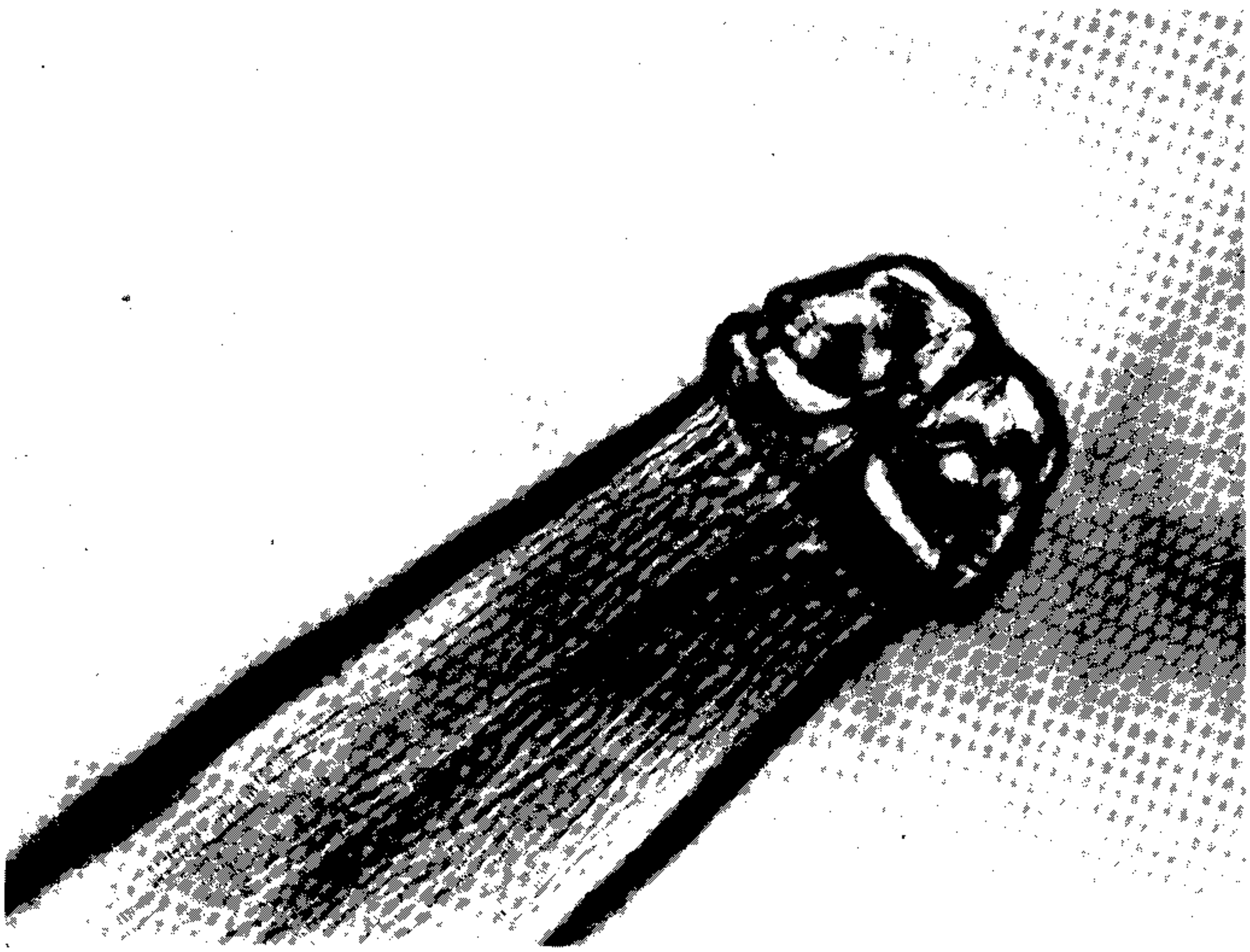

Fig. 8: anterior extremity of adult *Lagochilascaris minor*, from the upper respiratory tract of a cat, 2 months PI. Mounted in glycerol, 200 x.

ynx, pharynx, and rhinopharynx. One cat sacrificed 6 weeks PI had a fistula in the posterior wall of the pharynx. Another cat showed, after a similar time of development, destructive lesions at the base of the tongue, forming sacs with inflamed walls containing mucus and eggs, with adult worms moving in and out (Figs 7, 8).

No cat infected by oral administration of eggs, or fed mice inoculate 3 days previously, showed any eggs in the feces, or any larvae or adult worms upon necropsy.

DISCUSSION

The establishment of a heteroxenous cycle for an ascarid nematode capable of infecting man, as well as its transfer to easily maintained laboratory animals, has both theoretical and practical significance. In addition to a simple model for the study of the biology of this parasite and its host relationships, it provides a valuable tool for teaching.

Sprent (1954) described the encystment of larvae of *Ascaris columnaris* in mice and the later development of the adults in raccoons was described by Tiner (1949), this being the first determination of a heteroxenous cycle of development in a terrestrial ascarids. For the genus *Lagochilascaris* Leiper, 1909, a heteroxenous cycle was described by Smith et al. (1983) for *L. sprenti,* in rodents and didelphid marsupials, these latter being the definitive host, where the adult worms were found in tunnels below the gastric mucosa. In the rodents, the larvae were encysted in the skeletal muscles, and in 16.4% of the rodent laboratory infections, adult worms had developed in this tissue.

In the mouse, *L. minor* develops in a manner similar to *L. sprenti,* with some differences. After distribution by the blood stream, and final implantation in the skeletal muscle, adipose and connective tissue, the larvae grow rapidly, occasionally changing site, and in some 4 weeks, attain a stage like that described by Sprent (1971) as advanced 3rd stage. During a period of up to various months PI, the larvae show a characteristic filiform posterior prolongation, which is surrounded by a spherical thickening. This structure is displaced caudally

by the preferential growth of the posterior part of the developing larva. Incidentally, some of the larvae moult, and the sheaths show this structure clearly, but there is no trace of the post-cephalic furrow that can be observed in the following stages. The population of the 4th stage larvae is small in the mouse, and we have not observed adults in this host.

The frequent observation of two superimposed sheaths upon larvae spontaneously hatched within the stomach of the rodents indicates that *L. minor,* as with other ascarids (Araujo, 1972), has 2 intraovular moults, and that the infective stage is the 3rd. Thus the larvae hatching in the rodent host are the infective early 3rd stage, while those that infect the definitive host are advance.

Further study of this model would be necessary, since the course of the parasitosis in cat, similar to that in man, suggests the possibility of autoinfection as an explanation of the long period chronicity (Mondragon et al., 1973; Moraes et al., 1985). The finding of adult worms and embryonated eggs in the cavity lesions in the cat, and the lack of embryonated eggs in the feces of these animals, at least suggests the possibility of autoinfection.

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