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Studies on the Site of Development of Slow Loris *(Nycticebus coucang)* Filaria in Mosquitoes

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Introduction

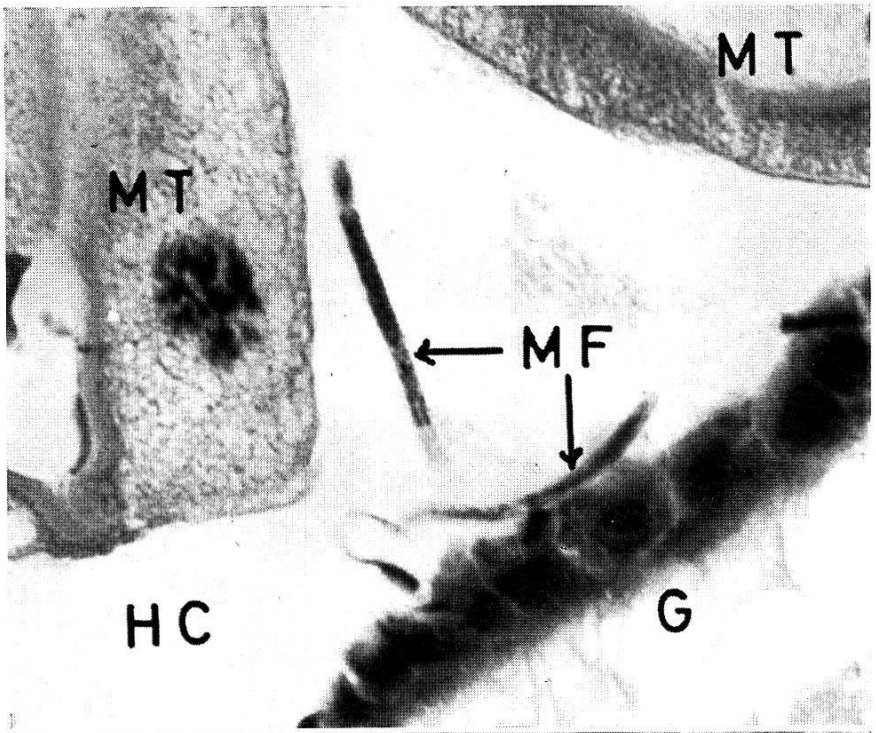
Filariasis in the loris *(Nycticebus sp.)* was first reported by MATHIS & LEGER (1909) from Indo-China. In a later study, PETTER (1958) redescribed the adult worms from *Nycticebus tardigradus* which according to her had come from Calcutta. However, no work was reported on the life cycle of this parasite, until the publication by DUNN & RAMACHANDRAN (1962) in which the morphology of the microfilariae and its vector transmission were described. We have also studied the life cycle of this parasite, and observations on the vector susceptibility and larval morphology are being published elsewhere (ZAMAN & CHELLAPAH, 1968). In this paper, the site of development of the filaria in the insect host based on the serial sections of mosquitoes, is being presented.

Material and Methods

One naturally infected slow loris showing a microfilaraemia of about 500 mf per 20 cmm was used in these experiments. The mosquito species was *Armigeres subalbatus* (Coquillet) (= *A. obturbans* auct.). In the previous study we have found that this species is highly receptive to slow loris filaria (ZAMAN & CHELLAPPAH, 1968). The mosquitoes were reared from an established laboratory colony which had been maintained in the Department (BARR & CHELLAPPAH, 1964). The slow loris was anaesthetized by intraperitoneal injection of sodium barbital and then introduced into the mosquito cage containing 5–6 days old unfed mosquitoes. After feeding, which occurred almost immediately, the mosquitoes were removed and separated into different containers. These mosquitoes were then fixed in formal saline or Bouin's at intervals of 5 minutes, 1/2 hour, 4 hours, 6 days and 15 days, after the intake of the blood meal. The fixed mosquitoes were embedded in paraffin and/or celloidin according to the standard technique. The sections were made both in longitudinal and transverse planes and stained with haematoxylin and eosin.

Results

The time required for engorgement of the mosquitoes varied from 3 to 4 minutes. The sections made, 5 minutes after engorgement, showed that the microfilariae had already started to leave the mid gut to enter into the haemocoele. In a few sections, it was possible to see the actual process of penetration of the mid gut wall by the microfilaria. Sections made after 1/2 hour, showed almost complete absence of microfilariae in the gut lumen, the majority having escaped into the haemacoele (Fig. 1). During this stage many of the microfilariae were found to be concentrated in the posterior part of the abdomen. The sections made after 4 hours showed most of the microfilariae lying inside or in the vicinity of the fat cells of the abdomen. In some sections microfilariae were seen apparently penetrating the fat cells. Occasionally a whole microfilaria could be seen coiled in a distended fat cell (Fig. 2). Sections made, 6 days after blood meal, showed a very high concentration of developing larvae within the fat cells



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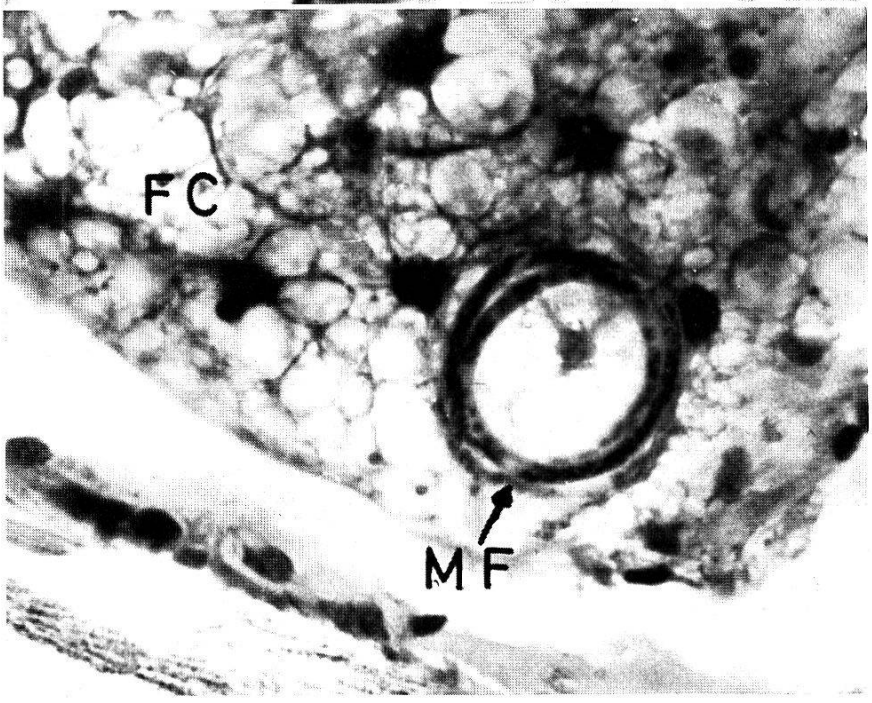

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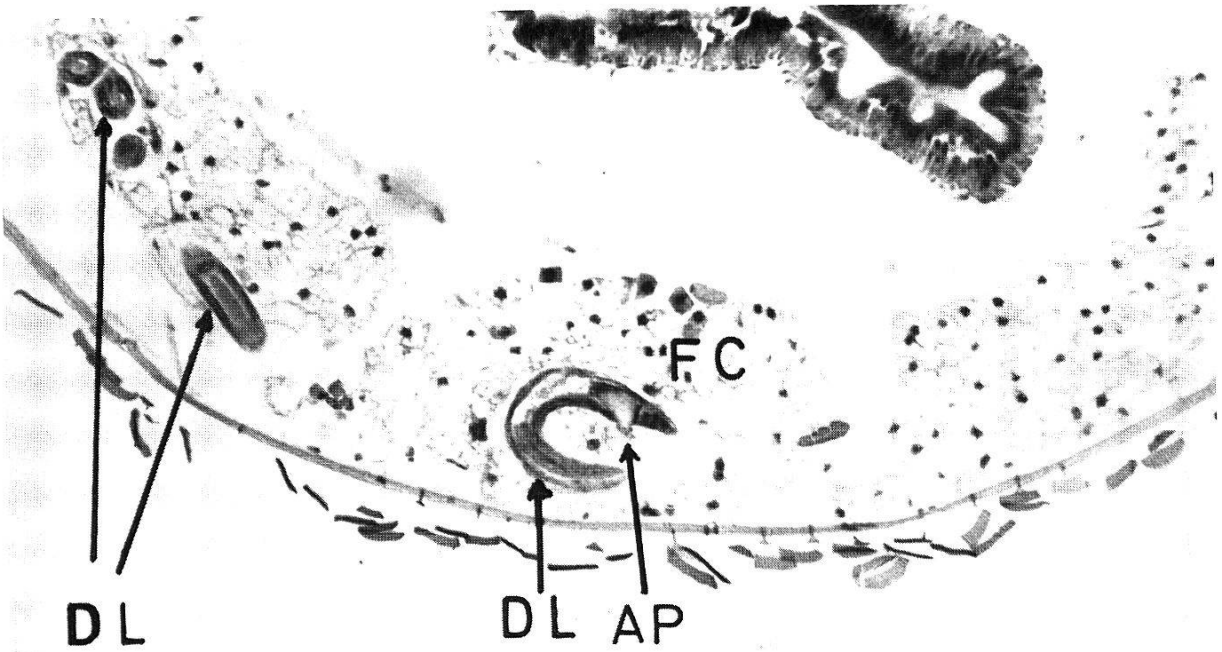
Fig. 1. Showing two microfilariae lying in the haemocoele after escaping from the gut. × 400. G: Mosquito gut, MF: Microfilaria, MT: Malphigian tubules, HC: Haemocoele.

Fig. 2. Showing a microfilaria lying coiled in a distended fat cell. × 400. MF: Microfilaria, FC: Fat cells.

of the abdomen (Fig. 3). By this period, larvae had developed into the second stage and the anal plug had formed (Fig. 3). In a few instances developing larvae were also seen in the fat cells of the thorax (Fig. 4) and the fat cells of the head (Fig. 5). Then they will develope into the third stage. Sections made 15 days after blood meal showed the highest concentration of the infective larvae in the head region and the mouth parts (Fig. 6). In some mosquitoes of this



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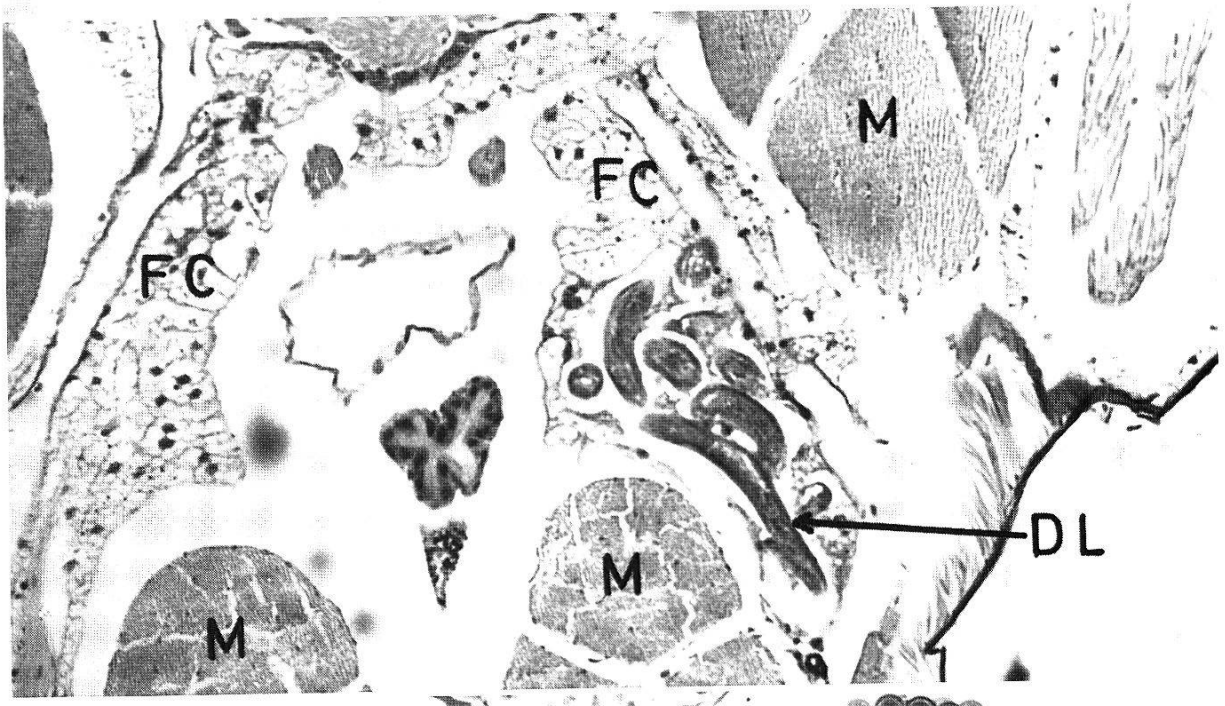

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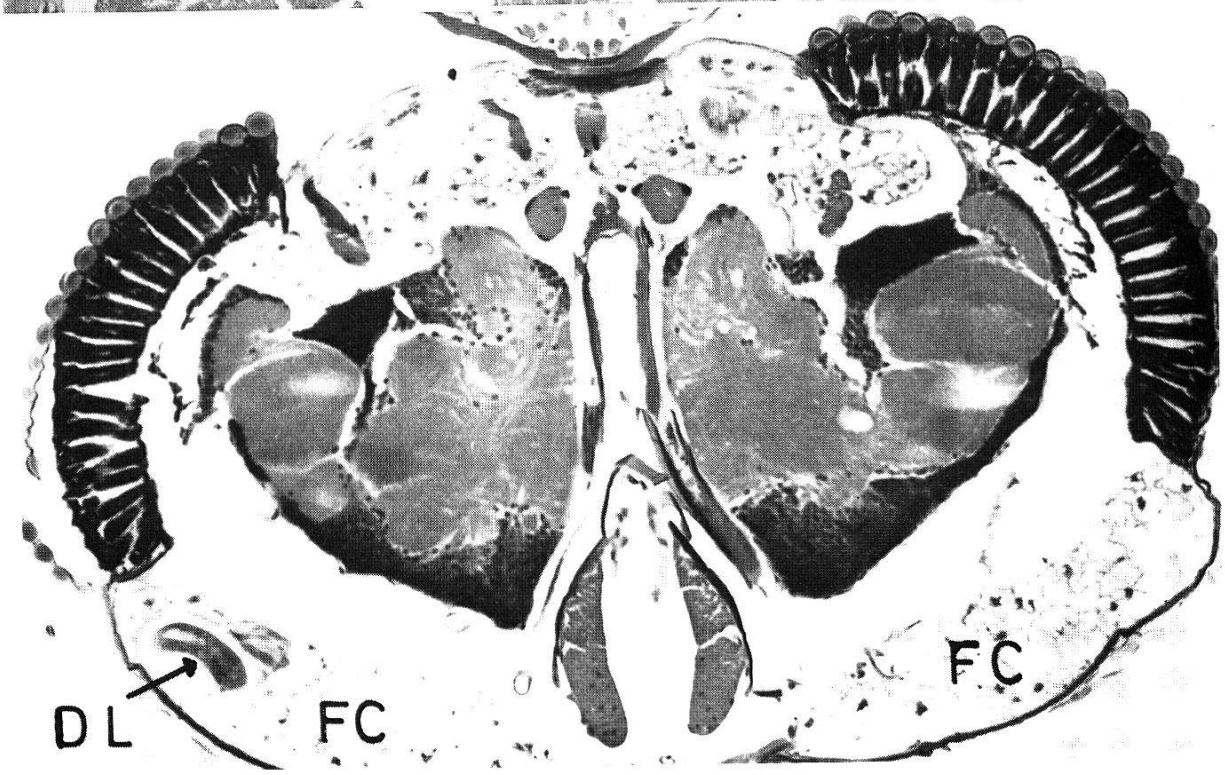

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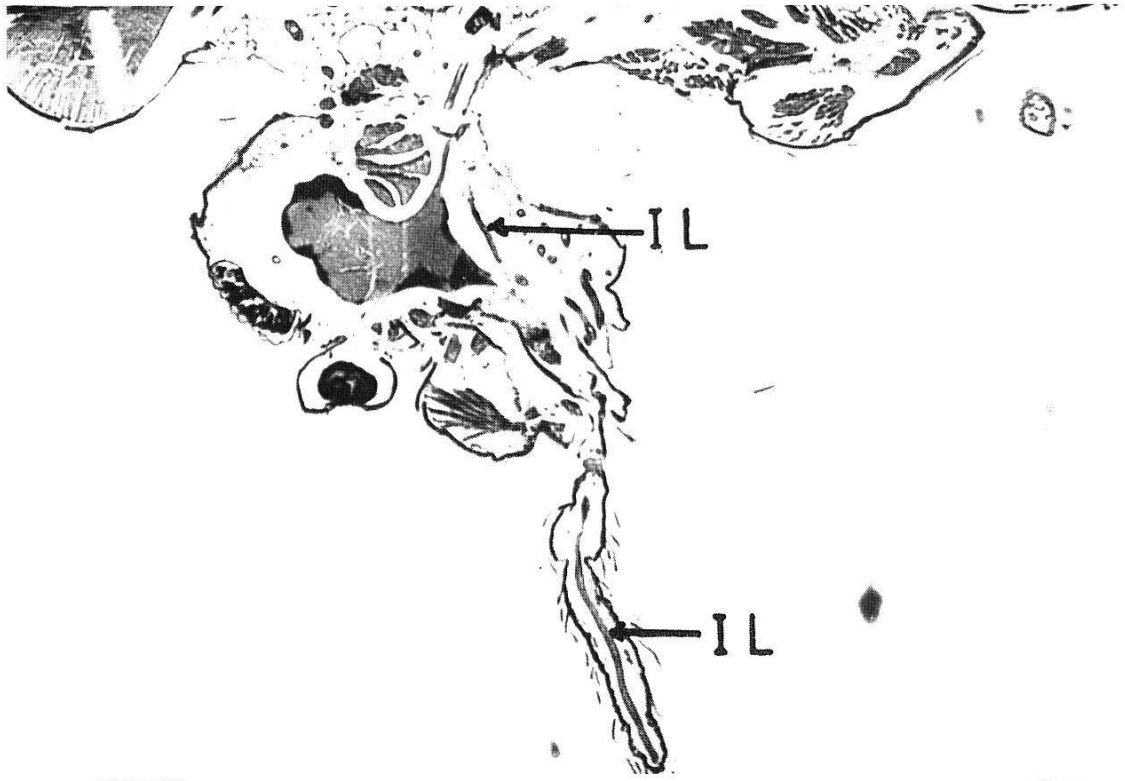

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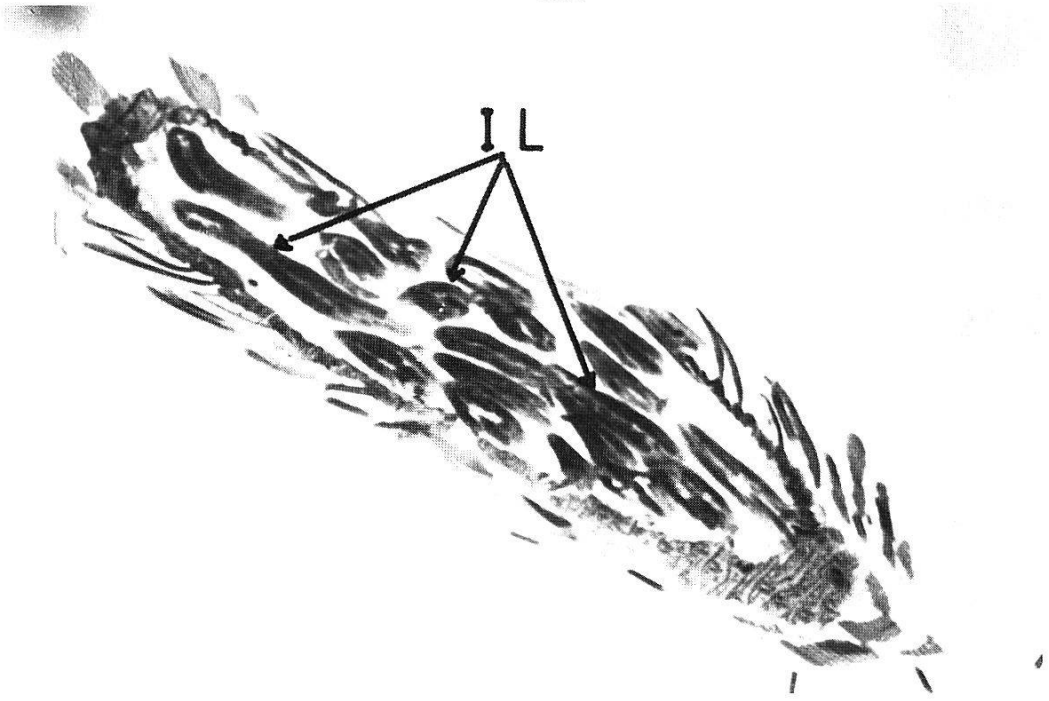

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Fig. 3. Showing developing larvae in the fat cells of the abdomen. × 100. DL: Developing larvae, AP: Anal plug.

Fig. 4. Showing developing larvae in the fat cells of the thorax. × 100. DL: Developing larva, FC: Fat cells, M: Muscles.

Fig. 5. Showing developing larvae in the fat cells of the head. × 100. DL: Developing larva, FC: Fat cells.

Fig. 6. Longitudinal section of the head region and the mouth parts showing infective larvae. × 40. IL: Infective larvae.

Fig. 7. Showing a large number of infective larvae in the labium. × 100. IL: Infective larvae.

batch the labium was almost completely packed with the third stage or the infective larvae (Fig. 7).

Discussion

Filarial larvae are known to display a high degree of specificity with regard to tissues in which they develop in the arthropod hosts. The various sites which have been described include the muscles, fat body, malphigian tubules and the haemocoele. Our findings show that the slow loris filaria develops exclusively in the fatty tissues of the mosquito concerned. Although the abdominal fat cells were the main sites of development occasionally larvae were seen developing in the thoracic and cephalic fat. Unlike *Brugia* and *Wuchereria* species, these parasites did not enter the muscles of the mosquito at any stage of the development. The entry into the haemocoele occurred only temporarily when the microfilariae left the mid gut on their way to the fat cells and later, after the completion of the larval development, during their entry into the mouth parts.

Other filarial worms which are definitely known to develop in the fat cells of the mosquito are *Oswaldofilaria chlamydosauri* (Breinl) which according to MACKERRAS (1953) develops in the fatty tissues of *Culex fatigans* and *Culex annulirostris* Skuse and *Dipetalonema arbuta* which according to HIGHBY (1943) develops in the fatty tissues of an *Aedes* species and *Mansonia pertubans*. In addition there are a few indefinite reports of the development of the larval stages of *Dirofilaria aethiops*, *Foleyella brachyoptera* and *Foleyella dolichoptera* in the connective tissues or the fat cells of mosquitoes by WEBBER (1955); CAUSEY (1939) and KOTCHER (1941). None of these publications, however, clearly illustrate with photomicrographs, the actual process of development in the fatty tissues of the mosquitoes. The only previous publication which clearly shows the developing larvae in the fat cells is by LAVOIPIERRE (1958), but in this case the sections are not of the mosquito but of *Chrysops silicea* showing the development of *Loa loa*.

Conclusions

The site of development of the slow loris filaria was studied in *Armigeres subalbatus*. The serial sections of the infected mosquitoes were made at intervals of 5 minutes, 1/2 hour, 4 hours, 6 days and 15 days. It was found that the larval development occurred exclusively in the fatty tissues of the mosquito. The abdominal fat was mainly involved but occasionally the larvae were found developing in the thoracic and the cephalic fat.

Acknowledgements

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