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**ELECTRON MICROSCOPIC STUDIES ON THE MORPHOLOGY  
OF MICROFILARIAE OF *DIROFILARIA IMMITIS*  
I OBSERVATIONS OF THE WHOLE BODY**

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Morphological observations on microfilariae of *Dirofilaria immitis* living in the blood of dogs affected with heart filariasis have been conducted by several workers<sup>1-7)</sup>, but their observations were by optical, phase-contrast or ultra-violet microscope.

In the present work, the microfilariae obtained from the peripheral blood of dogs affected with the disease have been studied by electron microscopic methods in order to elucidate their morphological structure more fully. In particular the structure of the body surface of the microfilaria will be described in this paper.

**MATERIALS AND METHODS**

1) Materials

The blood containing microfilariae was obtained from dogs in the veterinary hospital of the Hokkaido University diagnosed clinically as having heart filariasis. The microfilariae in the blood plasma were washed two times by centrifugation with phosphate-buffered physiological saline solutions (pH 7.4) following which they were suspended in a small amount of the same solution. The suspended solution was mounted on seat meshes with collodion membranes strengthened by a coating of carbon powders. After 10 minutes, the solutions on the seat meshes were sucked up gently with blotting paper leaving the microfilariae on the surface of the seat meshes. The seat meshes were introduced into a small chamber saturated with osmic acid gas for 10 minutes where fixation of the microfilariae on the meshes took place. After fixation the seat meshes were recoated with carbon powders.

The samples prepared in this manner were then examined.

2) Method of examination

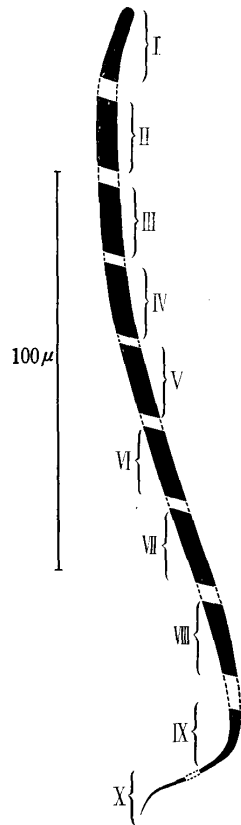
The holes in the seat meshes were about 100  $\mu$  in diameter. The microfilariae were much longer than this so that they overlapped several holes. Hence microscopic examination of some areas of the microfilariae bodies were disturbed by framework of the some holes.

Because the entire microfilariae could not be seen under a visual field, it was very difficult to determine the exact position of the bodies except by their heads and tails. However serial tracings using these reference points enabled entire body to be depicted.

The body was divided into 10 parts as shown in figure 1 and observations of each part

were described. Electronmicroscopic photographs were taken of numerous microfilariae, but the values used were the mean values of 5 microfilariae photographed clearly from the tops of their heads to the ends of their tails.

FIGURE 1  
Whole body traced by  
serial observations



## RESULTS

The form of the head is obtuse or club-like. The width of the body at the apex is  $2.3 \mu$ , whereas the width of the body  $20 \mu$  from the head is about  $4.7 \mu$ .

No special structures are found on the head except on the side where striations at regular intervals of about  $1.0 \mu$  are found.

From the 2nd through the 8th part, the widths of the body and the interval of the striations are relatively similar, as shown in the table. The widths of the body and interval of striations range from  $4.7 \sim 5.3 \mu$ , and from  $0.9 \sim 1.3 \mu$ , respectively.

TABLE Widths of bodies and intervals of striations

PART OF BODY	BODY WIDTH	INTERVAL BETWEEN STRIATIONS
1st	$2.3 \sim 4.7 \mu$	$1.0 \mu$
2nd	5.0	1.3
3rd	5.3	1.3
4th	5.3	1.2
5th	5.3	1.2
6th	4.7	1.2
7th	4.7	1.0
8th	4.7	0.9
9th	$2.7 \sim 1.2$	$0.7 \sim 0.4$
10th	$0.8 \sim 0.3$	0.4

On the 9th part, the width of the body decreases proximally from  $2.7 \mu$  to  $1.2 \mu$  distally, while at the same time the interval of the striations decreases from  $0.7 \mu$  proximally to  $0.4 \mu$  distally.

On the 10th part, the striations on the body surface are so clear that they look like peculiar body-joints. The width of the body  $10 \mu$  from the tail is  $0.8 \mu$  and the interval between striation is  $0.4 \mu$ . At a distance of  $3.3 \mu$  from the end of the tail, the body takes a straight, smooth rod-like shape with a width of  $0.3 \mu$ . The end of the tail is blunt.

For this part, the electron density was very low in comparison with the other parts.

## DISCUSSION

It has been reported by TAYLOR<sup>5,6)</sup> that there are numerous transverse grooves  $0.6 \mu$  in width on the cuticle and that there is a small hook-like structure

at the tip of the head of the microfilaria of *Dirofilaria immitis*. In our observations using the electron microscope, the hook-like structure was not found at the head, and the striations were found on the entire body with the exception of the tip of the head and the tail.

These striations are especially clear on the surface of the posterior part of the body where they look like body-joints. The width between the striations varies but, in general, it is about  $1.0\sim 1.3\mu$  from the head to the middle part of the body, and about  $1.0\sim 0.4\mu$  distal to that.

NEWTON & WRIGHT and WALLENSTEIN & TIBOLA reported that the straight form of the tail of the microfilariae of *Dirofilaria immitis* was characteristic of the species and by this findings the microfilariae were differentiated from the microfilariae of tissue filariid—*Dipetarlonema* sp.—having a curved, button hook-like tail.

The straight tail of the microfilariae of *Dirofilaria immitis* was confirmed by our observations.

#### SUMMARY

Using the electron microscope, the structure of the body surface of microfilariae of *Dirofilaria immitis* obtained from dogs with heart filariasis was observed. The width of the striations on the body surface and the shape of the tail were described.

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#### EXPLANATION OF FIGURES

- Fig. 2 The head  $\times 3,000$   
Fig. 3 Striations on the body surface of the 1st part  $\times 15,000$   
Fig. 4 The 4th part of the body  $\times 3,000$   
Fig. 5 Striations on the body surface of the 4th part  $\times 15,000$   
Fig. 6 The 8th part of the body  $\times 3,000$   
Fig. 7 Striations on the body surface of the 8th part  $\times 15,000$   
Fig. 8 The 10th part (tail) of the body  $\times 30,000$

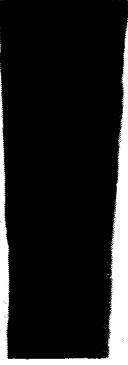
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