THE OCCURRENCE OF MICROFILARIA OZZARDI IN PANAMA[1]

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In August, 1930 an expedition from the Gorgas Memorial Laboratory, Panama, R. de P., headed by Dr. Herbert C. Clark, found unsheathed microfilariae in 6 of 295 blood films taken from natives residing in villages of the Tuira River basin, Darien Province, Panama. On a subsequent expedition to Darien in April, 1931, Dr. Clark collected blood films from 333 natives and 55 Indians from the same locality and found microfilariae in the blood of 4 (1.2 per cent) of the natives and in 17 (30.9 per cent of the Indians. At the suggestion of Dr. Clark, the writer, during July, 1932, made another survey for microfilariae in Darien and found an infection rate of 44.5 per cent among 119 Indians and 9.9 per cent among 244 natives examined. The larva is identified as *Microfilaria ozzardi*, a parasite which has not previously been reported from Panama.

Microfilaria ozzardi was first described by Manson (1897) from blood films collected by Ozzard from Indians living in the interior of British Guiana. Later studies showed that this larva was identical with a microfilaria found by Manson (1895) in the blood of natives of St. Vincent, British West Indies, and described as *M. demarquayi* (1897). The adult forms of the parasite were first found by Daniels (1899) in British Guiana. The species has recently been assigned to the new genus Mansonella by Faust (1929).

[1] This investigation was aided by a grant from the Rockefeller Foundation.

The writer is indebted to Dr. Herbert C. Clark, Director of the Gorgas Memorial Laboratory, for suggesting the investigation and for the use of the data from his two previous surveys.

Besides British Guiana and St. Vincent, *M. ozzardi* has also been reported from St. Lucia (Galgey, 1899) and Dominica (Low, 1902) in the British West Indies and from Dutch Guiana (Bonne, 1920), and recently Vogel (1927) has shown that the microfilaria, *M. tucumana*, reported as occurring very extensively in northern Argentina (Araoz and Biglieri, 1914; Mühlens et al., 1925) is identical with *M. ozzardi*. Also Balfour (1921) reported having found a microfilaria identical with *M. demarquayi* (= *M. ozzardi*) in the blood of a native in the Atrato River valley, Colombia, during a trip through that country in 1915.[2] The present report indicates that there is a region of heavy endemic infection with this filaria in southeastern Panama, which also probably extends into the adjacent part of Colombia.

Microfilaria ozzardi is a sheathless larva, much smaller than *M. bancrofti*, and is characterized by a sharp-pointed tail free of nuclei at the tip. The size is variable depending largely on the method of fixation but is usually given as about 200μ by 5μ. According to Vogel (1927) the location of the various points of identification in relation to the total body length of the larva is as follows:

	per cent
Beginning of the first head nuclei	2.5
Nerve ring	21.9
Excretory pore	31.5
Excretory cell	35.0
G_1 cell	69.3
G_4 cell	79.2
Anal pore	79.4
End of the last tail nuclei	98.0

The microfilariae found in Panama were studied in the living condition and in dried thick blood films, dehemoglobinized and stained with Giemsa and with iron haematoxylin. The morphological details of the larvae fitted the description of *M. ozzardi* and the measurements fell within the limits of variation of the

[2] The writer's attention has been called to a recent report by Hoffman (1930) on the occurrence of two cases of infection with *Microfilaria ozzardi* in the interior of Yucatan, Mexico. (Annales del Instituto de Biologia de la Universidad Nacional Antonoma de Mexico, *1*, 55–57.)

species. The average length of 20 specimens in dried blood films stained with Giemsa was 185μ, and the location of the principal land-marks in identification was as follows:

	per cent
Beginning of the first head nuclei	2.2
Nerve ring	22.2
Excretory pore	30.9
G_1 cell	67.9
Anal pore	79.0
End of the last tail nuclei	98.2

The minute cephalic stylet described for *M. ozzardi* was seen in only a few specimens and was not visible in the ordinary fixed preparations. The prepuce at the anterior end, however, was sometimes discernible in the stained specimens.

MICROFILARIA OZZARDI IN DARIEN PROVINCE

The province of Darien, which is located in the southeastern part of Panama next to the Colombian border, is for the most part uncleared jungle country sparsely inhabited by natives of negro blood, and by Indians who have retained their racial purity and most of their original customs and ways of living. The largest part of the province is drained by the Tuira River and its tributaries (see accompanying map, fig. 1), which afford practically the only means of travel. The natives for the most part live in villages along the lower stretches of the rivers while the Indians live mainly in detached houses further upstream.

Examination for the microfilariae was made in ordinary thick blood films consisting of several drops of blood taken from the lobe of the ear, dried and stained in dilute Giemsa, the entire film being examined under the low power of the microscope. Covering the stained film with a thin layer of oil was found greatly to increase visibility. The large majority of infections consisted of only a few parasites per slide, usually less than 10 and many times only 1, but there were a few cases in which the number ranged up to several hundred. In many cases a second blood film taken at the same time as the first was also examined. All blood films were taken during the day time, mainly between the hours of 10 a.m. and 5 p.m.

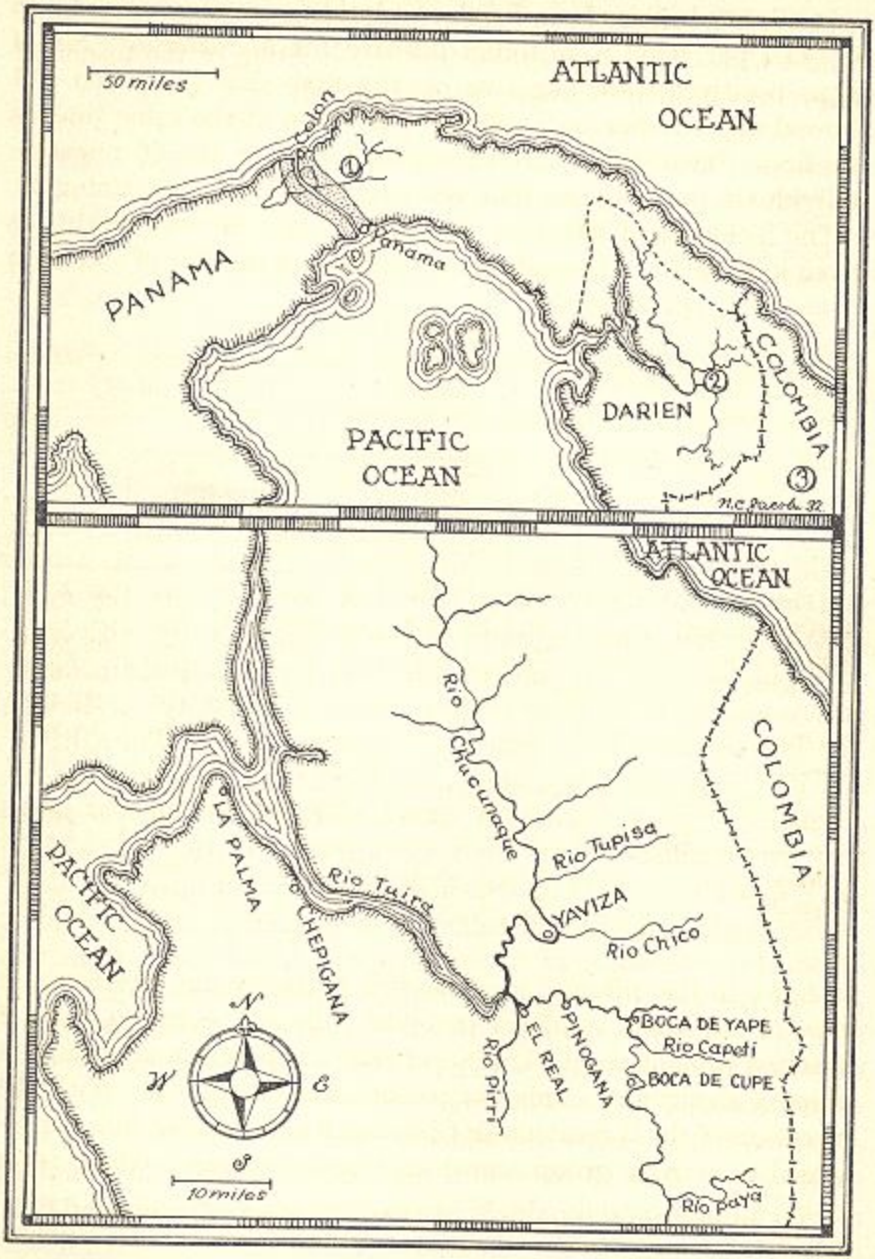

FIG. 1. *Above:* Map of Panama showing location of Darien Province. (1) Chagres River valley; (2) Tuira River valley; (3) Atrato River valley. *Below:* Detailed map of Tuira River basin, Darien Province.

In all, 119 Indians from 7 different localities were examined and 53 (44.5 per cent) were found positive for *M. ozzardi*.[2] Six of these positives were negative on the first slide examined but showed microfilariae on a second slide taken at the same time as the first. Two slides were examined for 34 of the 66 negative individuals, but only one slide was available for the remaining 32.

The incidence of infection according to age, sex and locality is given in table 1. Although some variation in the rate of infection

TABLE 1

Incidence of Microfilaria ozzardi among Darien Indians according to age, sex and locality

LOCATION	NUMBER	AGE GROUPS									TOTAL		
		Less than 6 years			6 to 15 years			Over 15 years					
		+	−	Per cent +	+	−	Per cent +	+	−	Per cent +	+	−	Per cent +
Rio Pirri	44	2	8	20	4	6	40	17	7	71	23	21	52
Rio Chucunaque	24	1	8	11	1	2	33	5	7	42	7	17	30
Boca de Yape	28	1	8	11	3	9	25	6	1	86	10	18	36
Rio Chico	16	2	1	67	2	1	67	6	4	60	10	6	63
Rio Tupisa	3							2	1	67	2	1	67
Rio Paya	3				0	1	0	1	1	50	1	2	33
Rio Capeti	1							0	1	0	0	1	0
Total	119	6	25	19.4	10	19	34.5	37	22	62.7	53	66	44.5
Males	73	4	15	21	7	10	41	25	12	68	36	37	49
Females	46	2	10	17	3	9	25	12	10	55	17	29	37

is shown in the different localities it is probably not significant in view of the small numbers involved. In any event, a rate of infection ranging from 30 to 60 per cent was found in all groups in which a significant number of persons was examined. With the exception of the 3 individuals from the Rio Paya, the Indians belonged to a tribal group known as Choco Indians. The 3 from

[2] The writer wishes to thank Dr. P. W. Wilson, Lieutenant Commander (M. C.), United States Navy, and Mr. Charles Martin, Chief Pharmacists Mate, United States Navy, for their many courtesies while this survey was made, and also for permission to use their data on the presence of microfilariae in the native villages.

Rio Paya belonged to the Cuna tribe which is of essentially the same racial stock.

It will be noted that the incidence of infection was appreciably higher in the 2 older age groups as compared with the children under six years of age. This undoubtedly reflects the longer time that the older people have had to acquire the infection and possibly also the time necessary for the parasite to develop. It was also noted that all of the 9 persons with comparatively heavy infections (more than 50 microfilariae per slide) were in the group over fifteen years of age. Another difference is noted when the figures are tabulated according to sex. In all 3 age groups the

TABLE 2

Incidence of Microfilaria ozzardi among Darien Natives

LOCATION	AUGUST, 1930			APRIL, 1931			JULY, 1932		
	Number	Number +	Per cent +	Number	Number +	Per cent +	Number	Number +	Per cent +
El Real	143	4	2.8	130	2	1.5	116	3	2.5
Pinogana	47	0	0	85	1	1.1	49	1	2.0
Boca de Cupe	105	2	1.9				23	13	57.0
Yaviza				118	1	0.8	28	1	3.6
Boca de Yape							28	6	21.4
Total	295	6	2.0	333	4	1.2	244	24	9.9

incidence of infection is somewhat higher among the males than among the females; but the difference is much more marked in the 2 older age groups than in the children under six years of age.

In addition to the Indians, 244 natives from 5 villages in Darien were examined. The incidence of *M. ozzardi* is given in table 2 in comparison with the surveys made by Dr. Clark in August, 1930 and April, 1931. In most instances the rate of infection is quite low, less than 3 per cent. The exceptions are the natives at Boca de Cupe and Boca de Yape examined in July, 1932. The infection rate of 57 per cent found in the former locality is surprisingly high in view of the incidence of only 2 per cent found two years previously.[4] The natives examined at Boca de Yape, who

[4] A private communication from Dr. Wilson says that this rate of infection has been maintained in additional individuals examined in this village.

showed an incidence of 21 per cent, were living in close proximity to Indians in whom the rate of infection was 36 per cent. The natives in the other localities in general lived under much the same conditions as the Indians except that there was a much larger cleared area around their houses.

Opportunity was had to reëxamine 3 persons found positive for microfilariae by Dr. Clark in August, 1930 and 2 others who were positive in April, 1931. All 5 of these individuals were found to be still positive in July, 1932.

TABLE 3

Numbers of Microfilaria ozzardi in the blood of two infected persons examined at intervals during a 24-hour period

DATA	TIME	NUMBER OF MICROFILARIAE IN 40 CU. MM. OF BLOOD	
		Case 1	Case 2
1932			
August 8	10 a.m.	75	59
	2 p.m.	26	196
	6 p.m.	15	3*
	11 p.m.	8	12
August 9	6 a.m.	10	15
	11 a.m.	18	12

* Blood taken from finger—all others taken from lobe of ear.

Note: The counts were made by taking up the blood in a graduated pipette, taking in 20 times the volume of 0.5 per cent acetic acid, and examining the entire amount under the low power of the microscope.

It is also interesting to note that the 2 youngest children found infected with *M. ozzardi* were 14 and 16 months old respectively. These were both native babies, one at Boca de Yape and the other at Boca de Cupe. Nine other children under two years of age were negative.

M. OZZARDI IN THE CHAGRES RIVER VALLEY

As a part of a malaria control study, about 500 blood films from individuals living in the Chagres River valley, provinces of Panama and Colon, are examined every month at the Gorgas

Memorial Laboratory. During the period January to August, 1932, 10 persons were found to harbor microfilariae in the blood. The writer had the opportunity of examining the slides from 5 of these individuals and found the parasite to be *Microfilaria ozzardi*. All of these infected persons were Colombians who had moved from the Atrato River valley to Panama within the last year. The Atrato River valley lies directly across the divide from the endemic center of *M. ozzardi* in Darien Province, Panama. As yet no autochthonous infections with *M. ozzardi* have been found in the Chagres River valley.

Through the coöperation of Dr. Clark opportunity was had to make examinations at intervals during a twenty-four hour period of the blood of 2 of the infected individuals from the Chagres River valley. The results of these examinations are shown in table 3. Although there was considerable variation in the number of microfilariae, there was no evidence of a marked periodicity, the larvae being found at all times when an examination was made. This result is in keeping with those of observers in other localities who have also failed to find any periodicity in the occurrence of *M. ozzardi* (Fülleborn, 1908, etc).

DISCUSSION

No evidence has yet been found that *Mansonella ozzardi* is in any way pathogenic. The adult worms according to Daniels (1899) are to be found in the connective tissue surrounding the peritoneal cavity in contrast to those of *Wuchereria bancrofti* which localize in the lymphatics. In the present study none of the individuals infected with *M. ozzardi* showed any obvious symptoms which could be ascribed to the filarial infection.

Although *Mansonella ozzardi* is apparently of no significant public health importance, it is interesting to speculate on the spread of the parasite in Panama. From the data available it would appear that the center of infection is among the Indians and that the parasite is spreading from them to the natives in the villages along the upper part of the Tuira River. The much higher incidence of the parasite in Boca de Cupe in July, 1932, as compared with August, 1930, indicates that the rate of infection

among the natives is increasing. It is noteworthy that this village is further up the Tuira River than any of the other native villages. In the course of malaria surveys no cases of infection have so far been found in Chepigana and La Palma, two villages near the mouth of the Tuira River. It will be interesting to see if the area of endemic infection spreads to these villages, and also if the presence of introduced cases of *M. ozzardi* in the Chagres River valley will result in the establishment of the parasite in this part of Panama.

The transmission of *M. ozzardi* has not as yet been worked out. Several observers, most recently Davis (1928), have obtained partial development in mosquitoes, but it seems probable that some other type of biting insect is the vector. In this connection it is significant that all of the localities in which *M. ozzardi* occurs are heavily forested areas.

SUMMARY

1. *Microfilaria ozzardi* was found in the blood of 44.5 per cent of 119 Indians examined in the Tuira River basin, Darien Province, Panama.

2. The infection rate in native villages in the same region varied from 2 to 57 per cent and averaged 9.9 per cent in the 244 natives examined.

3. A few cases of infection with *M. ozzardi* were found in the Chagres River valley, Panama, but all of these were recent arrivals from the Atrato River valley, Colombia.

4. No evidence was found that *M. ozzardi* is pathogenic or in any way a significant problem of public health importance.

5. Southeastern Panama is an endemic center of infection with *M. ozzardi* heretofore unknown in the geographical distribution of the parasite.

REFERENCES

(1) Araoz and Biglieri: (Cited by Davis, N. C., Amer. Jour. Hyg., 1928, vii, 457–466), 1914.

(2) Balfour, A.: War Against Tropical Disease, London, 1921, p. 66.

(3) Bonne, G.: (Cited by Vogel, Abhandlungen aus dem Gebiete der Auslandskunde, Hamburger Universität, XXVI, Rheihe D. Medizin (Festschrift Nocht), 1927, ii, 573–584), 1920.

(4) Daniels, C. W.: Brit. Med. Jour., 1899, i, 1459.
(5) Davis, N. C.: Amer. Jour. Hyg., 1928, vii, 457–466.
(6) Faust, E. C.: Human Helminthology, Philadelphia, 1929, p. 468.
(7) Füllleborn, F.: Arch. f. Schiffs u. Trop. Hyg., 1908, Beihefte 9.
(8) Galgey, O.: Brit. Med. Jour., 1899, i, 145–146.
(9) Low, G. C.: Brit. Med. Jour., 1902, i, 196–197.
(10) Manson, P.: Brit. Med. Jour., 1897, ii, 1837.
(11) Mühlens, P., Dios, R. L., Petrocchi, S., and Zuccarini, J. A.: (Cited by Davis, N. C., Amer. Jour. Hyg., 1928, vii, 457–466), 1925.
(12) Vogel, H.: Abhandlungen aus dem Gebiete der Auslandskunde, Hamburger Universität, XXVI, Rheihe D. Medizin (Festschrift Nocht), 1927, ii, 573–584.

PLATE 1

Microphotographs of Microfilaria ozzardi in Thick Blood Films Stained with Giemsa

Figure 1, × 100; figures 2, 3 and 4, × 970. *N*, nerve ring; *EP*, excretory pore; *AP*, anal pore; *T*, tip of tail.

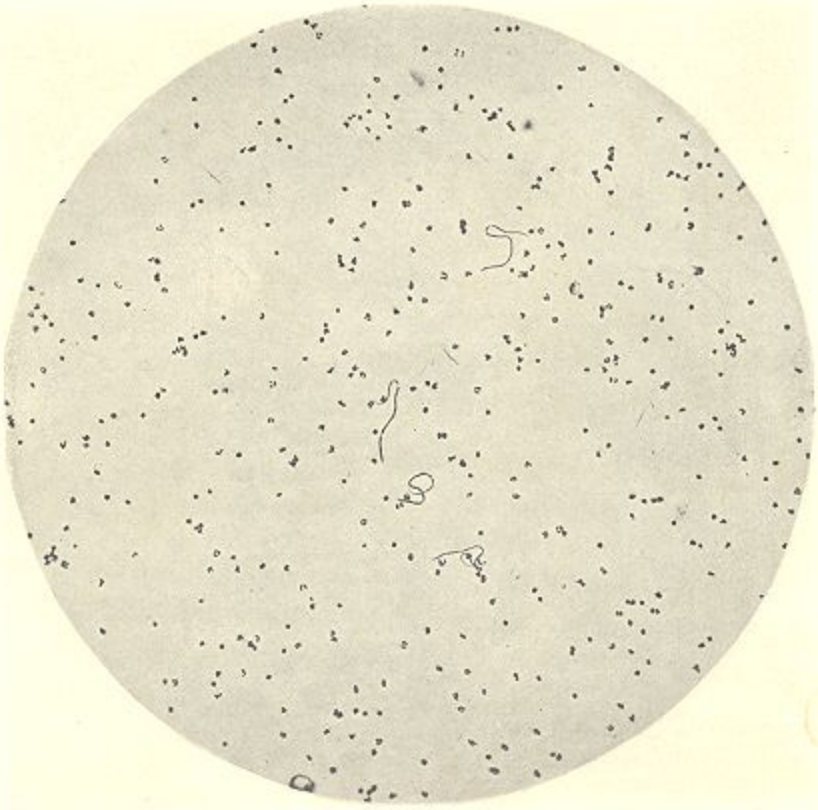

FIG. 1

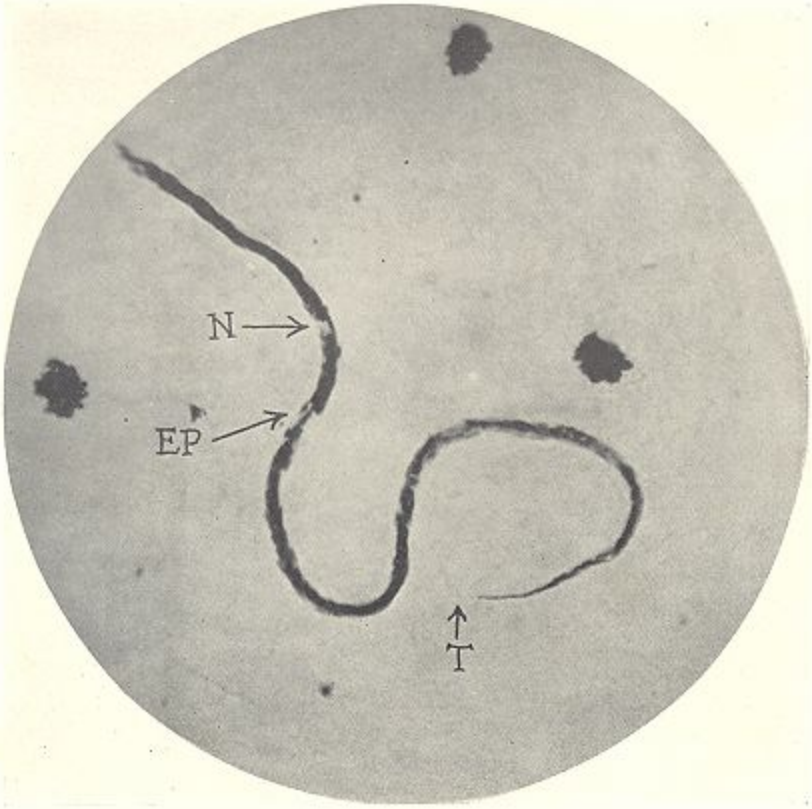

FIG. 2

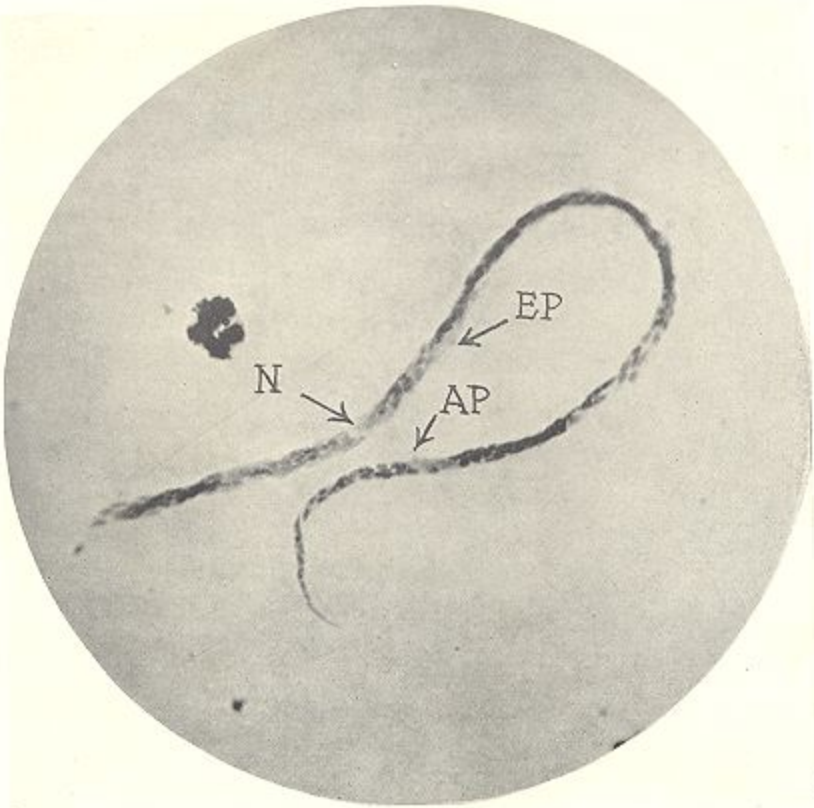

FIG. 3

FIG. 4