Limited fly load and development of *Onchocerca volvulus* microfilariae in Guatemalan *Simulium ochraceum*†

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ABSTRACT

Fly load of *Onchocerca volvulus* microfilariae and their early development in *Simulium ochraceum* were examined at intervals of 10, 30 and 60 min after ingestion and then hourly up to 72 hours. Many microfilariae ingested were damaged by the cibarial armature of the vector, limiting the fly load, but some of these damaged microfilariae were able to penetrate the gut before they died. Thus, the mean number of the living parasites per fly in the head and thorax decreased gradually with elapse of time after ingestion. The parasites ingested by *S. ochraceum* did not develop beyond the microfilarial stages during the first 24 hours; thereafter only thoracic forms (1st-stage larvae) were found to be alive. The high mortality of the infected fly might be caused by the high intakes of microfilariae and also by normal and/or partly wounded microfilariae invading various organs. The numbers of microfilaria ingested from different biting sites were usually high in comparison with the densities observed in each site; this indicates that the microfilariae might be attracted by the biting females.

INTRODUCTION

In the endemic area of Guatemalan onchocerciasis, many investigators have recognized several species of black-flies, *Simulium* spp. as potential vectors of the disease. Of these *S. ochraceum*, *S. metallicum* and *S. callidum* have been found to be the more important potential vectors, the first being considered the most important, followed by the second and third (DALMAT, 1955; DELEON & DUKE, 1966; GARMS, 1975; COLLINS, 1979 a).

OMAR & GARMS (1975) showed that many *Onchocerca volvulus* microfilariae were destroyed by the cibarial armature of *S. ochraceum* during ingestion, thereby limiting the mortality of the vector caused by superinfection. On the other hand, they also stated that the armature was not recognized in *S. metallicum*, which had a high rate of microfilarial intake and consequently a high mortality. The teeth of certain mosquitoes also damaged other microfilariae (COLUZZI & TRABUCCI, 1968). Recently MCGREEVY *et al.* (1978) mentioned that the microfilariae of *Wuchereria bancrofti* and *Brugia pahangi* were killed by the chewing action of the cibarial and pharyngeal armatures and by other papillae and spines in the fore-gut of mosquitoes. Moreover, discussing the damage to ingested microfilariae of *O. volvulus*, DUKE & LEWIS (1964) emphasized the importance of the peritrophic membrane in limiting the number of microfilariae reaching the thoracic muscles of *S. damnosum*. BAIN *et al.* (1974) observed that only 1 to 2% of the total numbers of microfilariae ingested by *S. ochraceum* reached the haemocoel.

The present study was designed to determine the intake of and damage to *O. volvulus* microfilariae by *S. ochraceum* and to observe their early development in the

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vector maintained under the natural conditions of a Guatemalan endemic focus of onchocerciasis.

MATERIALS AND METHODS

The study area: The study was done from April to June, 1978 in three coffee plantations with different onchocerciasis endemicity. Two of the plantations, Finca Santa Monica Ivone and Finca Valle de Oro were in the Department of Suchitepequez, and the other, Finca Peña Blanca, in the Department of Escuintla, Guatemala, located on the Pacific slopes of the Sierra Madre. The areas were about 700 m to 850 m above sea level.

Skin biopsy on the volunteers: All the volunteers were positive for *O. volvulus* microfilariae in their skin unless otherwise mentioned. A Holth type sclerocorneal punch was used for the biopsy. The skin snips taken were incubated in 0.85% NaCl for one hour at room temperature; thereafter the microfilariae (mf) which emerged were counted under an electric compound microscope. The microfilarial skin density (mfd) was expressed as the number per mm² of skin of the patients in the present study.

Black-fly collection and maintenance: All the black-fly collections were made on the infected residents in the endemic area, except one uninfected person who agreed to participate as a normal human bait. Most fly catches were done from 08.30 to 11.00 hr in the morning. The flies captured were satiated with a blood meal, satiation taking about 5 min. A proportion of the flies captured was maintained in a small cage (15 × 10 × 10 cm) covered with nylon mesh, into which about 15 or 20 flies were released in mass; the cage was kept in a large box (80 × 50 × 50 cm). These flies were exposed to the natural conditions of the endemic focus (Finca Valle de Oro) in the shade. They were fed by dipping the cotton wick in 10% sucrose solution.

Dissection of flies: A sample of the flies collected was dissected immediately where caught at intervals of minutes or hours, to determine the total intakes of microfilariae and the migration in the fly. When examined after minutes, the intact blood meal was removed separately to a drop of 0.85% NaCl, while the remaining thorax, head and legs were carefully dissected in a drop of the solution on the same slide. With the intact blood meal from each fly, the samples were divided into several lots because of the difficulties in counting accurately the number of microfilariae ingested. All the samples were mounted on a slide with a cover-slip which was pressed slightly to rupture the small pieces of fly tissue. They were then examined under the microscope with × 100 or 400 magnification. Since many microfilariae were destroyed by the cibarial armature of the fly during ingestion, it was mainly the typical tail parts which were marked and counted, eliminating other fragments of microfilariae. The microfilariae with only a small cut (less than about 10 µm) on the tail parts were counted as normal; thus very small fragments of the tail were also eliminated. When the microfilariae showed even slight activity under × 400 magnification, they were recorded as alive, even those with partly damaged body parts. Only undamaged normal microfilariae and larvae were measured under the microscope with a micrometer. To obtain information on the microfilarial intakes by the vector in relation to the different biting sites (torso, forearm and legs) on the volunteers, a proportion of the flies captured was dissected after storage overnight in a refrigerator. In this case, the slides with microfilariae were air dried, fixed for 5 min with 100% ethanol and stained with Giemsa solution.

RESULTS

Three observations at intervals of minutes were made, dissecting a total of 20 flies 10 to 60 min after ingestion, as shown in Table 1. The results indicated a total of 829.9 mf intakes, of which 20.85% (173.0 mf) were listed as living microfilariae throughout the examination. Most of the living microfilariae were, however, found to be partly damaged, showing only sluggish movement even in the haemocoel and other organs. Almost all the living microfilariae in the gut had been wounded by the cibarial armature of the fly.

Seven observations were made during the period three to 72 hours after ingestion, 53 flies being dissected. As shown in Table 2, living microfilariae were observed until 24 hours after ingestion but most were wounded and inactive; this indicates that wounded microfilariae can survive for a long time in the flies. At three hours, a total of 715 microfilariae had been ingested by the fly, but only 4.81% of them had escaped from the gut to the thorax and head. During the period 30 to 72 hours, only thoracic forms (first-stage larvae) were recognized alive, indicating a mean number per fly ranging from 0.7 to 3.1 in each examination, average 1.8; many parasites were found to be dead in the abdomen, thorax and head of the fly. The dead microfilariae in the gut during this period were like waste thread, allowing counting under high magnification of the microscope.

FIG. 1. Survival of *S. ochraceum* after engorgement on infected (A) and uninfected (B) persons and of flies without blood meal (C).

TABLE 1

No. dead mf

TABLE 2

No. dead mf or larvae

*Including gut and haemocoel.

Table 3 shows the early development of *O. volvulus* in the flies exposed to natural conditions (21 to 32 °C with 43 to 98% R.H.). The parasites did not develop beyond the microfilarial stages during the first 24 hours after ingestion under these field conditions. After 30 hours, the *O. volvulus* larvae were recognized as first-stage in the flies, showing a gradual increase in width and decrease in length.

Survival of three groups of captured flies were compared under natural conditions, 30 which had not sucked blood, 40 which had engorged on an uninfected normal volunteer and 142 which had engorged on an infected person with mfd 8.3. The results are shown in Fig. 1. The survival rate of the group engorged on the infected person decreased rapidly during the first three days but only gradually in the remaining two groups. The flies which had not sucked blood had a higher survival rate than those which had engorged on a normal person.

The microfilarial intakes from different biting sites are shown in Table 4. The four participants, A, B, C and D had variable mfd. From these human baits, a total of 48 flies were captured and dissected to determine the microfilarial intakes from the respective biting sites. The results indicated some correlation between mfd in each site and microfilarial intake, though some abnormally high intakes were observed from the forearm of patient A. The mean number of microfilariae ingested by *S. ochraceum* was usually higher, in almost all cases, than the mfd of the respective anatomical sites.

TABLE 3

Early development of *O. volvulus* larvae expressed as length and width in *S. ochraceum* at selected intervals of time ingestion

Time after ingestion	No. measured	Stage	Measurements*	
			length	width
0	20	mf**	265.5 ± 9.44	6.4 ± 1.28
10 min	15	mf	260.7 ± 13.85	6.4 ± 0.99
15 hrs	14	mf	287.1 ± 13.26	6.9 ± 0.95
24 hrs	2	mf	275.0 ± 21.20	8.3 ± 1.06
30 hrs	11	1st	231.8 ± 30.93	11.0 ± 2.36
48 hrs	10	1st	202.0 ± 24.86	16.6 ± 4.14
72 hrs	18	1st	206.7 ± 40.87	24.7 ± 6.75

*Fresh specimens expressed as mean in μm with standard deviation.

**Skin microfilariae from a patient.

TABLE 4

Microfilarial skin densities and numbers of the microfilariae ingested by *S. ochraceum* collected from different biting sites in each infected person with *O. volvulus*

Patients	Mean mfd per mm ² skin			Mean no. of mf per fly ingested		
	torso*	forearm**	leg**	torso	forearm	leg
A	48.6	2.7	1.0	34.6 ± 31.2 (11)	2771 (1)	1.6 ± 1.2 (4)
B	34.7	0	0.3	—	6.3 ± 8.4 (3)	1.4 ± 2.0 (4)
C	16.8	6.3	0.2	43.8 ± 40.1 (8)	21.5 ± 17.0 (4)	—
D	14.6	12.9	0.7	55.1 ± 44.9 (8)	94.6 ± 3.8 (2)	5.1 ± 8.2 (3)

*Two skin biopsies each, 4 in total, were taken from both shoulder and buttock sites, respectively.

**One skin biopsy each was taken from the right and left extremities.

***Mean numbers with standard deviation; the figures in parentheses indicate the number of flies dissected.

DISCUSSION

In the current study, many microfilariae of *O. volvulus* were taken up by *S. ochraceum* engorging on an infected person. Most of them, however, were destroyed and/or wounded by the cibarial armature of the fly. Consequently, only a few microfilariae escaped from the gut to the haemocoel, thorax and other parts. Microfilariae which migrated died gradually with the elapse of time after ingestion. It was noted that some of these dying individuals could invade and disperse in organs other than the gut of the fly. During the first 24 hours considerable numbers of living microfilariae were found alive in the abdomen, partially damaged with, for example, a small cut on the tail; thereafter none were recognized alive in the abdomen. This shows that wounded microfilariae can survive for a while after ingestion by *S. ochraceum*. DUKE & LEWIS (1964) suggested that the peritrophic membrane of *S. damnosum* limited the number of microfilariae reaching the thoracic muscles, 44% of the total intake migrating into the muscles and 91% of them developing to the infective stages. In the same vector, moreover, *O. volvulus* microfilariae with a low developmental potential disappeared during the first two days after ingestion, and did not develop beyond the microfilarial stages (DUKE, 1962).

It is also important to pay attention to the dying first-stage larvae in the thorax during 30 to 72 hours. COLLINS *et al.* (1977) observed in three groups of flies with average microfilarial intakes of up to 12.5, 24.2 to 44.7 and 51.1 to 117.8 per fly that microfilariae produced an average of 0.002 infective larvae per surviving fly in the first group, 0.12 to 0.12 in the second and 0.51 to 1.69 in the third. Moreover, COLLINS (1979a) also stated that 1.42% of Guatemalan *S. ochraceum* had developing infections (First- or second-stage larvae) and 0.31% had infective larvae. On the other hand, the figures for *S. damnosum* from an area in the Bong Range, Liberia were 17.5 and 2.6 respectively (GARMS, 1973). It thus appears that, the former species is an inefficient vector compared to the latter, because *S. ochraceum* is not easily or heavily infected by *O. volvulus* even when fed on persons with a high mfd (COLLINS, 1979a; COLLINS *et al.*, 1979). In the present work, the average number of first-stage larvae per surviving fly ranged from 0.7 to 3.1, average 1.8, during the period 30 to 72 hours after ingestion. According to GARMS (1975), 90 (2.92%) of the 3087 examined were positive for first- and/or second-stage larvae, although only four (0.13%) out of the total contained third-stage larvae, in various endemicities of Guatemalan onchocerciasis. In contrast to these results, in African *S. damnosum* the mean numbers of *O. volvulus* larvae remained fairly constant from the third day onwards until infective larvae were produced at seven to eight days (DUKE, 1962).

The development of *O. volvulus* in *S. ochraceum* has been investigated by several workers under laboratory conditions (DELEON & DUKE, 1966; COLLINS, 1979b; COLLINS *et al.*, 1977; MATSUO *et al.*, 1980). In nature, however, the conditions are unstable in each endemic area of onchocerciasis. In Central America, the endemic areas of the disease are situated on a mountainous southern slope; in Guatemala the disease is distributed between 700 m and 1,700 m above sea level (TADA *et al.*, 1979). In these endemic areas, the climate changes are great and would stress or stimulate developing larvae of *O. volvulus* in the vector, *S. ochraceum*. Under the natural conditions in this study, the parasite did develop beyond the microfilarial stages during 24 to 30 hours after ingestion. At 30 hours, all the larvae observed were first-stage, measuring, on average, 231.8 µm long and 11.0 µm wide. Under laboratory conditions, the sizes of

larvae in *S. ochraceum* were 190.0 by 8.8 μm at 48 hours (COLLINS, 1979 b) and 181 to 231 μm long on the third day (MAISUO *et al.* 1980).

The high mortality of the flies engorged on the infected person was caused by the invasion of normal and/or partly wounded microfilariae into various organs of the fly. Even though the present vector, *S. ochraceum*, has a well-developed cibarial armature (OMAR & GARMS, 1975), considerable numbers of microfilariae can survive for a time, even when wounded; some damaged microfilariae were able to penetrate the gut before dying.

In a comparison of microfilarial intakes with the mfd in different biting sites, the vector tended to take in more microfilariae than appeared in the skin. In one case (patient A), abnormally high numbers (2771) of microfilariae were ingested by a single fly from the forearm with mfd of 2.7. Similar cases were frequently experienced in our field examinations of microfilarial intakes by the vector (unpublished data). The results indicate that the microfilariae may be attracted by the biting females and that the number of microfilariae ingested may be very high in comparison with the densities observed in the human skin (WHO, 1976).

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