

Duration of blood feeding of *Simulium ochraceum* in relation to intake of *Onchocerca volvulus microfilariae*

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| journal or publication title | 衛生動物 = Medical entomology and zoology |
| volume | 31 |
| number | 3 |
| page range | 209-214 |
| year | 1980-09-15 |
| URL | http://hdl.handle.net/2297/11694 |

Duration of blood feeding of *Simulium ochraceum*
in relation to intake of *Onchocerca volvulus*
microfilariae¹⁾

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(Received: April 17, 1980)

Abstract: Intake of microfilariae of *Onchocerca volvulus* by *Simulium ochraceum*, the main vector of onchocerciasis in Guatemala, was studied. The initiation of intake of microfilariae was around 30 sec after landing. Thereafter, the number of microfilariae taken by flies increased as the feeding time increased. After 3 to 4 min of landing, 69% of flies finished their blood meal, and the intake of microfilariae reached the highest. Thereafter, increase of feeding time did not increase the intake. With the volunteers of moderate density of microfilariae (55-116 Mf per 10 mm²), the number of microfilariae taken by the flies was correlated with the density of microfilariae in human skin. With a volunteer of very low density (1.8 Mf per 10 mm²), however, an extraordinarily high intake of microfilariae was observed. This suggested the possibility of the flies attracting or stimulating the microfilariae in the skin of low density carriers.

INTRODUCTION

Dalmat (1955) reported that main anthro-

philic black fly species in Guatemala were *Simulium ochraceum*, *S. metallicum* and *S. callidum*, and many studies have been done on the biology of these three species in Guatemala. Garms (1975) reported in his preliminary study on the quantitative aspects of *Onchocerca volvulus* transmission that, of wild-caught flies belonging to the above-mentioned three species, only *S. ochraceum* had harboured the third stage larvae of *O. volvulus*. Our studies in Onchocerciasis Control Project in Guatemala (unpublished) also show the similar results regarding this problem, although two infective larvae, indistinguishable from *O. volvulus*, were recently found in the head of wild-caught *S. metallicum*. Garms and Ochoa (1979) confirmed recently the predominant role of the highly anthropophilic species *S. ochraceum* as vector of this disease. Collins (1979) also reported that *S. ochraceum* was the only significant vector. Based on these findings, *S. ochraceum* is now considered to be a main

¹⁾ This study was supported by the Ministry of Public Health, Republic of Guatemala and by the Japan International Cooperation Agency (JICA). (GJCRCPO-MENSAP series No. 28).

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vector of onchocerciasis in Guatemala.

A lot of studies on the intake of *O. volvulus* microfilariae (Mf) have also been done, and the relationships between Mf intakes and their development in the flies were reported (De Leon and Duke, 1966; Bain *et al.*, 1974; Bain, 1976; Collins *et al.*, 1977; Omar and Garms, 1975).

The present studies were done to determine the initiating time of Mf intake by flies and the relationship, if any, between feeding time and Mf intake.

MATERIALS AND METHODS

The experiments were carried out in August, September and December 1977, at Finca Monica Ivoné, a cardamon and coffee plantation, situated in the Department of Suchitepéquez, Guatemala.

Wild flies of *S. ochraceum* were caught after complete or partial blood meal on volunteers with Mf of *O. volvulus* in their skin. In order to know microfilarial density (MfD) of volunteers, skin snips were taken with a sclerocorneal punch from both sides of the shoulders prior to the biting experiments. The shirts of the volunteers were cut off 10 cm × 10 cm at the back near a left shoulder blade, in order to restrict the biting to the exposed skin. After the collection, skin snips were taken from three or five points of the exposed skin, *i.e.* three at random in August and September and one each from four corners and one from a center in December. Thereafter, the snips were incubated for 60 min in saline solution and the numbers of Mf were counted. The size of biopsies ranged 3.1 to 10.3 mm². The geometric mean Mf skin densities were 1.3 in the volunteer with the lowest MfD and 131.6 in the highest per 10 mm² of a biopsy.

Feeding studies were divided into two parts; limited feeding and free feeding. In the former studies, flies were removed from the skin with a small plastic tube at 1/2, 1, 2 and 4 min after landing. In the latter studies, when the fly satiated and detached from the skin, it move up into the tube, which was then captured. Majority of the flies which came to bite the volunteers was *S. ochraceum*, and only this species was

studied.

Flies were anesthetized with chloroform soon after catching and the size of the abdomen was measured. Then they were dissected under a microscope to check parity and to count the number of microfilariae in gut. Amount of blood ingested was also estimated, according to the four categories; (1) no blood ingested (—), (2) only detectable by dissection (±), (3) distinct in appearance (+) and (4) full in gut (⊕). When microfilariae were found damaged, presumably due to a buccopharyngeal armature as reported by Omar and Garms (1975), a fragment with a pointed end was counted to be one microfilaria.

RESULTS

Distribution of Mf in the surface of the exposed body

The results are shown in Table 1. Biopsies were taken twice on successive 2 days from each of four volunteers, except No. 2 who was not available on the first day.

Marked variations of MfD were observed among five sites of the exposed skin of the same volunteer, and daily fluctuation was also considerable. For instance, in the volunteer No. 3, the average number of Mf on the first day (95.3) was more than five times than that of the second day (18.3).

Blood ingestion and change of abdomen size

In the experiments of limited time feeding, 30 (73.2%) of 41 flies did not take blood in the first 30 sec. After one minute of the landing, 26 (59.2%) of the 44 flies took detectable blood and 15 (34.1%) took distinct blood. After 4 min, 29 (69%) of 42 flies took full blood meal and only 2 flies showed a slight ingestion (Table 2). Throughout the experiments, no flies changed their sucking sites, under the situation with no disturbance.

In the experiments of free feeding, the mean and median duration for satiation was 4'26'' and 4'00'' respectively, with the minimum of 1'35'' and the maximum of 8'35''. The number of flies satiated in the classified time duration after landing is shown in the first column of Table 4. It is interesting to

Table 1 Number of microfilariae of *Onchocerca volvulus* in different areas of exposed skin in shoulders of volunteers

| Volunteer No. Name | Date in 1977 | No. of Mf per 10 mm ² in the under-designated areas of exposed skin (10 cm × 10 cm) | | | | | Average (GM) |
|--------------------|--------------|--|-------------------|--------------------|--------------------|--------|--------------|
| | | Corner above-left | Corner below-left | Corner above-right | Corner below-right | Centre | |
| 1 M.R.C. | 13 Dec. | 5 | 3 | 5 | 0 | — | 2.5 |
| | 14 Dec. | 0 | 6 | 0 | 0 | 9 | 1.3 |
| | Ave. | | | | | | 1.8 |
| 2 M.J. | 13 Dec. | — | — | — | — | — | — |
| | 14 Dec. | 3 | 2 | 3 | 0 | 3 | 1.9 |
| | Ave. | | | | | | 1.9 |
| 3 S.F.S. | 13 Dec. | 121 | 168 | 48 | 315 | 25 | 95.3 |
| | 14 Dec. | 14 | 38 | 3 | 27 | 40 | 18.3 |
| | Ave. | | | | | | 42.1 |
| 4 D.S. | 13 Dec. | 209 | 64 | 59 | 52 | 263 | 101.8 |
| | 14 Dec. | 1,155 | 128 | 20 | 171 | 75 | 131.6 |
| | Ave. | | | | | | 115.7 |

Table 2 Blood ingestion in the experiment of limited feeding

| Feeding time (min) | No. of flies examined | Category of blood ingestion* | | | |
|--------------------|-----------------------|------------------------------|-------|------|------|
| | | — | ± | + | ‡ |
| 0.5 | 41 | 73.2% | 22.0% | 4.8% | 0 % |
| 1 | 44 | 4.5 | 59.2 | 34.1 | 2.2 |
| 2 | 44 | 2.2 | 11.4 | 70.5 | 15.9 |
| 4 | 42 | 0 | 4.8 | 26.2 | 69.0 |

* — : no blood sucked, ± : detectable by dissection,
+ : distinct in appearance but not full, ‡ : full in gut

note that the mean duration for satiation in parous flies was 5'10'', which was longer than that of nulliparous flies (4'08''), although the difference was not significant.

The size of abdomen after different sucking time is shown in Fig. 1. The abdomen began to enlarge its size around one or 2 min after landing. The enlargement in length was observed to cease after about 4 min, but the width continued to enlarge up to satiation.

Intake of microfilariae in the experiments of limited-feeding time

The number of flies with Mf and the mean number of Mf per fly in the experiments of limited feeding are shown in

Table 3.

Totally 41 to 44 flies were used in limited feeding test with seven volunteers, whose MfD ranged 1.8 to 115.7 in geometric mean per 10 mm² of skin. Three Mf were ingested by *S. ochraceum* after 30 sec of landing, one each by three flies, and 38 (93%) of 41 flies were negative. The number of Mf ingested by the flies increased, with the increase of feeding time, *i.e.* 0.07 in 30 sec-feeding, 6.3 in 1 min-feeding, 23.5 in 2 min-feeding and 61.9 in 4 min-feeding. The positive rate, or the number of flies with Mf per the number of flies tested, also increased, *i.e.* 7% in 30 sec-feeding, 79% in 1 min-feeding, 95% in 2 min-feeding and 100% in 4 min-feeding.

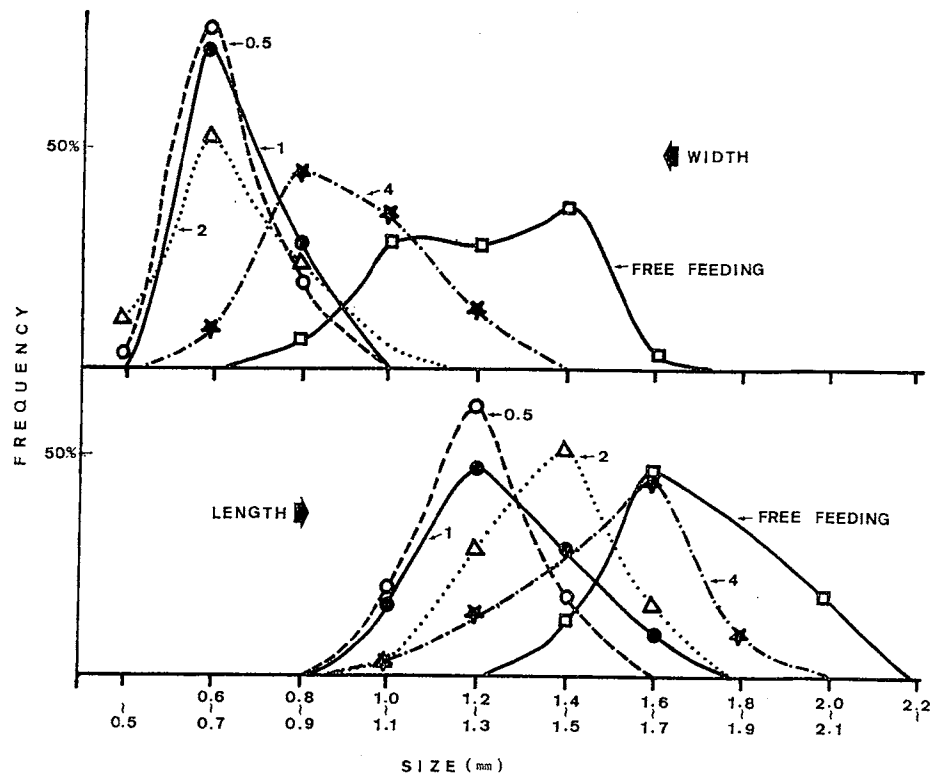


Fig. 1 Size of abdomen of *Simulium ochraceum* after the blood ingestion

Table 3 Number of microfilariae taken after limited time feeding of *S. ochraceum*

| Item | Duration of feeding in minutes | | | |
|------------------------|--------------------------------|-----|-------|-------|
| | 1/2 | 1 | 2 | 4 |
| Total No. of Mf taken | 3 | 277 | 1,033 | 2,958 |
| No. of flies used | 41 | 44 | 44 | 42 |
| No. of flies with Mf | 3 | 35 | 42 | 42 |
| Positive rate*(%) | 7 | 80 | 95 | 100 |
| Mean No. of Mf per fly | 0.07 | 6.3 | 23.5 | 61.9 |

* No. of flies with Mf/No. of flies tested

Intake of microfilariae in the experiments of free feeding

The results are shown in Table 4. All the flies used in the tests took one or more microfilariae, except one which was satiated in 1'35'', the shortest time of the whole experiment, on the volunteer M.M. (Mf density: 77.2 per 10 mm²). There was a tendency that flies which were satiated in shorter time within certain limit ingested less Mf.

The mean number of Mf per fly was 3.5 in 1-2 min-feeding and 19.0 in 2-3 min-feeding. It was as high as 62.6 in 3-4 min-

feeding. The numbers in the longer feeding than 4 min varied, ranging from 15.5 in 7-8 min-feeding to 130.0 in 8-9 min-feeding.

The relationship between density of Mf in volunteers and the number of Mf taken by flies, together with the feeding time of flies for satiation, is shown in Table 5.

The geometric mean of Mf per 10 mm² skin of the volunteer ranged 1.8 to 115.7. With the exception of volunteer No. 1, whose MfD was as low as 1.8, the Mf densities of six volunteers were moderately high, ranging from 42.1 to 115.7.

No clear relationship was observed be-

Table 4 Duration of sucking time required for satiation in freely-fed flies and number of microfilariae taken during the feeding

| Item | Duration of sucking time | | | | | | | |
|------------------------|--------------------------|-------|-------|-------|-------|-------|-------|-------|
| | 1'01" | 2'01" | 3'01" | 4'01" | 5'01" | 6'01" | 7'01" | 8'01" |
| | } | } | } | } | } | } | } | } |
| | 2'00" | 3'00" | 4'00" | 5'00" | 6'00" | 7'00" | 8'00" | 9'00" |
| No. of flies satiated | 2 | 5 | 18 | 6 | 7 | 4 | 2 | 1 |
| Total No. of Mf taken | 7 | 95 | 1,127 | 96 | 632 | 182 | 31 | 130 |
| Mean No. of Mf per fly | 3.5 | 19.0 | 62.6 | 16.0 | 90.3 | 45.5 | 15.5 | 130.0 |

Table 5 Density of microfilariae in the skin of volunteers, feeding time of *S. ochraceum* for satiation and number of microfilariae taken by the flies in free-feeding experiments

| Volunteer No. Name | MfD/10mm ² (GM) | No. of flies tested | Feeding time for satiation | | No. of Mf taken | | Index* |
|--------------------|----------------------------|---------------------|----------------------------|-------------|-----------------|--------|--------|
| | | | Mean | Range | Mean | Range | |
| 1 M.R.C. | 1.8 | 5 | 5'13" | 4'15"-6'55" | 31.4 | 10- 57 | 17.44 |
| 2 S.F.S. | 42.1 | 7 | 4'22" | 3'24"-5'53" | 166.7 | 94-203 | 3.96 |
| 3 A.M. | 55.3 | 8 | 4'26" | 2'45"-7'56" | 10.6 | 4- 22 | 0.19 |
| 4 I.R. | 69.0 | 3 | 6'09" | 4'00"-7'27" | 14.0 | 6- 18 | 0.20 |
| 5 M.M. | 77.2 | 9 | 3'10" | 1'35"-5'00" | 27.3 | 0- 54 | 0.35 |
| 6 F.S. | 78.7 | 10 | 4'34" | 3'10"-6'55" | 39.6 | 3-127 | 0.50 |
| 7 D.S. | 115.7 | 3 | 4'58" | 2'25"-8'35" | 69.0 | 10-130 | 0.60 |

* Index: (Mean No. of Mf taken)/(MfD/10mm²)

tween Mf density of volunteers and the feeding time of the flies for satiation.

In the group of No. 2 to No. 7 with moderate density of Mf, the mean number of Mf taken by the flies increased, as the Mf density of volunteers increased, except in the case of No. 2, in which comparatively high intake of Mf was observed. Volunteer No. 1 was quite exceptional. Although Mf density was very low, as high as 31.4 Mf were taken by the flies. This is clearly shown in the index, mean number of Mf taken divided by MfD per 10 mm². The index for No. 1 was 17.44, whereas the indices for the other six volunteers were low, ranging from 0.19 to 3.96.

DISCUSSION

In the endemic area of onchocerciasis in Guatemala, labourers in coffee plantations are the main victim for bite of the vector black flies. They used to hit flies landed on their bodies, as soon as they noticed it.

The flies which skillfully escaped the hit may come to human again, either to the same person or to the other one situated there. At any rate, the feedings of black flies are often disturbed by men under natural conditions.

Our study revealed that the intake of Mf by *S. ochraceum* initiated around 30 sec after landing, when blood ingestion was not observed yet in most of the flies. Majority of the flies finished their blood meal within 3 to 4 min after landing, if not disturbed. The intake of Mf by the flies increased, as the feeding time increased, up to 3-4 min after landing. Thereafter, however, the increase of feeding time would not increase the number of microfilariae taken.

When fed freely on human with moderate density of Mf in the skin, the intake of Mf by flies increased, as the Mf density of men increased. But the report of Collins *et al.* (1977) showed that no accurate relationship between the ingestion of microfilariae and MfD was obtained. Duke (1962) revealed

that, in densely infected cases with more than 150 Mf per mg of the skin, the intake of Mf declined. In our study, when MfD increased, the variation of the intake of microfilariae increased, probably because the Mf did not distribute uniformly, even in the skin as small as 10 cm×10 cm. It was also revealed that *S. ochraceum* took an extraordinarily high number of Mf from a volunteer with the lowest density of Mf in the skin. De Leon and Duke (1966) were forced to the conclusion in their experimental studies that the feeding Guatemalan *Simulium* spp. exerted some powerful attraction on the Mf of the Guatemalan strain. Our result also suggests the possibility of the flies attracting or stimulating the microfilariae in the skin of low density carriers during the blood meal.

ACKNOWLEDGEMENTS

We wish to acknowledge Drs. Juan Jose Castillo O., Horacio Figueroa M. and Hiroshi Takahashi for their encouragements throughout this study. Our thanks also due to Messrs. Yoichi Yamagata, M. M. Recinos C., E. L. Juarez, Oscar de la Lora and R. Sanchez who helped us in collecting specimens. Mr. P. A. Molina supervised our field works. We are also grateful to Drs. Kazuki Ogata and Takeshi Suzuki for their helpful criticisms in the preparation of this paper.

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摘 要

Simulium ochraceum における吸血時間と *Onchocerca volvulus* マイクロ フィラリアのとりこみ量

グアテマラにおけるオンコセルカ症の主要媒介種 *Simulium ochraceum* の *Onchocerca volvulus* 仔虫のとりこみに関して実験を行った。

仔虫のとりこみは、ブユが体表にとまってから30秒で始まった。3~4分後には65%のブユが吸血を完了し、とりこまれる仔虫数が最高に達した。それ以後は吸血時間がのびても、とりこみ量は増加しなかった。仔虫密度が皮膚10 mm²あたり55~116匹の中程度に感染した人にとっては、とりこまれる仔虫の数は皮膚中の仔虫の密度と関連していた。しかし仔虫密度が1.8と非常に低い人の場合に、異常に高い仔虫のとりこみが観察された。このことは、皮膚中の仔虫密度が低い場合には、仔虫がブユの吸血時に誘引あるいは何らかの刺激を受けることを示唆するものかもしれない。