

MICROFILARIAL DENSITY IN GUATEMALAN ONCHOCERCIASIS PATIENT'S SKIN WITH SPECIAL REFERENCE TO THE HOURLY INTAKE BY *SIMULIUM OCHRACEUM**

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Abstract: The present work was designed to assess the relationship between the hourly *O. volvulus* microfilarial density (mfd) in patient's skin and the hourly microfilarial intake by *S. ochraceum*. From two experiments employing 14 patients, it is suggested that there may be a little elevation of mfd during the period from evening through mid-night, but not pronounced. The microfilarial intake by the fly revealed two peaks, one in the morning and the other in the afternoon. Thus, the pattern of microfilarial intake did not correlate with the present diurnal pattern of mfd. The hourly intake was briefly discussed in relation to the biting activity and the parous rates of *S. ochraceum*. This report is the first case dealing with the hourly microfilarial intake by the black fly. Further such information would be required for a better understanding of the transmission mechanism of onchocerciasis in given endemic areas.

INTRODUCTION

The relationship between the microfilarial density in patient's skin and the hourly intake of *Onchocerca volvulus* microfilariae by *Simulium ochraceum* seems to have particular importance in the transmission of onchocerciasis in endemic areas. Recently, Campbell *et al.* (1980) working with Guatemalan onchocerciasis, stated that a nearly linear relationship was found between the mean skin mfd and the mean microfilarial uptake by *S. ochraceum* performing examination in the morning hours. Hitherto, there have been many works on the dermal microfilarial density (mfd) of onchocerciasis patients in connection with the biting activity of the vector, black fly, in endemic areas of the disease. In examinations at various times of the day, correlations between the peak mfd and biting activities of the vectors have been found in some geographical regions (Wegesa, 1966; Duke *et al.*, 1967; Lartigue, 1967; Thomas *et al.*, 1973; Duke & Moore, 1974; Anderson *et al.*, 1975). However, Picq and Jardel (1973), and Tada and Figueroa (1974) observed no diurnal periodicity in the mfd of *O. volvulus* in endemic areas of West Africa and Guatemala, respectively.

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Thus, the information on the diurnal periodicity of *O. volvulus* microfilariae in patient's skin still remains uncertain. In the light of transmission of the disease, however, it would be important to examine the intake of microfilariae by the vector, at various times of the day. Nevertheless, so far, little such an examination has been carried out. The microfilariae of *O. volvulus* may be attracted by the vector, black fly, when the flies are engorging on infected subjects (WHO, 1976; Hashiguchi *et al.*, 1981a); the number of microfilariae ingested may be very high in comparison with the densities observed in the human skin. It is assumed that the attracting condition, if any, may be influenced by times of the day in the given endemic area of onchocerciasis, since the black flies have a diurnal periodicity in their biting activity having two distinct peaks, one in the early morning and the other in the early afternoon (Collins *et al.*, 1981).

The present paper deals with hourly densities of the microfilariae in Guatemalan onchocerciasis patient's skin and hourly intake of *O. volvulus* microfilariae by *S. ochraceum*.

MATERIALS AND METHODS

The study area

The study was carried out from June 1978 through March 1979 in the following three coffee plantations with relatively high endemicity; *Finca* Santa Monica Ivone (Department of Suchitepequez), *Finca* San Rafael Sumatan and *Finca* Nimaya (Department of Chimaltenango) in Guatemala. They are located in the mountainous region 700 m to 800 m above sea level on the Pacific slopes of the Sierra Madre.

Skin biopsies on the subjects

Aiming at the assessment of microfilarial density in patient's skin, the skin biopsy was made at intervals of two to four hours, examining a total of 14 volunteers in the two study areas (*Finca* San Rafael Sumatan and *Finca* Nimaya). During night examinations, all the volunteers were permitted to sleep on bed, except the biopsy time. A Holth type sclerocorneal punch was used for the biopsy. Skin samples were taken from the left scapular region of the subjects approximately 0.5 cm away from the previous ones. The snips were incubated at 30 C in 0.85% NaCl for two hours and the numbers of microfilariae emerged were counted under a compound microscope at 40 \times magnification. The microfilarial density was expressed as the number per mm² of patient's skin.

Black fly collections

All the fly catches were made on the three infected adult male volunteers who wore the shirts with a 10 \times 10 cm grid at the back near the left scapula. The objective of the grid was to restrict the exposed skin for fly biting. In the three volunteers two skin snips each were taken from the exposed area; the mean rates obtained were used as mfd of the volunteers. The fly catches were performed from 0600 to 1800 of one day with hourly interval in *Finca* Santa Monica Ivone. All the

flies were captured after satiation with infected blood meal and then fixed immediately with 10% formalin and kept in the solution until dissection.

Dissection of flies

Since the migration of ingested microfilariae from stomach into other organs were prevented by above procedure, only abdominal parts of the fly were examined for the larvae. As the fixed flies with formalin had a coagulated blood meal in their stomach, they were treated in the laboratory as follows. The flies were washed with running water for two hours, incubated at 37 C in 0.5% KOH solution for 40 hours and then preserved in glycerin until examination. The abdominal region of flies was separated with insect needles under dissecting microscope, and then gently crushed with a slender teflon bar in a glass test tube with a drop of 0.85% NaCl solution. Care was taken to avoid any damage on the microfilariae. The materials were centrifuged at 1,000 rpm for 5 minutes; the supernatant was discarded, and Löffler's methylene blue solution was added to the sediments for the purpose of staining the microfilariae. All the samples were mounted on a slide with a cover-slip. 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, the typical tail parts of the microfilariae were marked and counted, eliminating other fragments of the larvae. The microfilariae with only a small cut (less than about 10 μ m) on the tail parts were counted as normal; thus very small fragments of the tail were also eliminated. The status of destruction in the present microfilariae ingested was similar to that found in our previous study in which the examination was carried out immediately after fly collections (Hashiguchi *et al.*, 1981b).

RESULTS

Microfilarial densities in the patient's skin

In order to assess the diurnal patterns of the skin density of *O. volvulus* micro-

Table 1 Microfilarial density in patient's skin, expressed as percentage per total mfd, in an endemic area, *Finca San Rafael Sumatan*, in Guatemala

Times	% mfd in each subject				Av. (%) \pm s. d.	Gm**
	No. 1 (59.6*)	No. 2 (180.4)	No. 3 (94.8)	No. 4 (20.8)		
0900	13.1	9.9	7.2	6.7	9.2 \pm 11.1	6.0
1200	24.2	12.0	7.3	2.9	11.6 \pm 12.2	6.0
1500	5.2	17.6	6.8	20.7	12.6 \pm 12.9	7.2
1800	10.6	22.1	7.0	9.6	12.3 \pm 13.1	7.6
2100	8.6	16.5	15.0	14.9	13.8 \pm 14.2	9.0
2400	19.1	5.5	19.1	9.6	13.3 \pm 13.5	8.0
0400	11.7	10.1	12.8	26.9	15.4 \pm 7.8	9.7
0600	7.6	6.3	25.0	8.7	11.9 \pm 8.8	6.8

* Total mfd/mm²/day, ** Geometric mean.

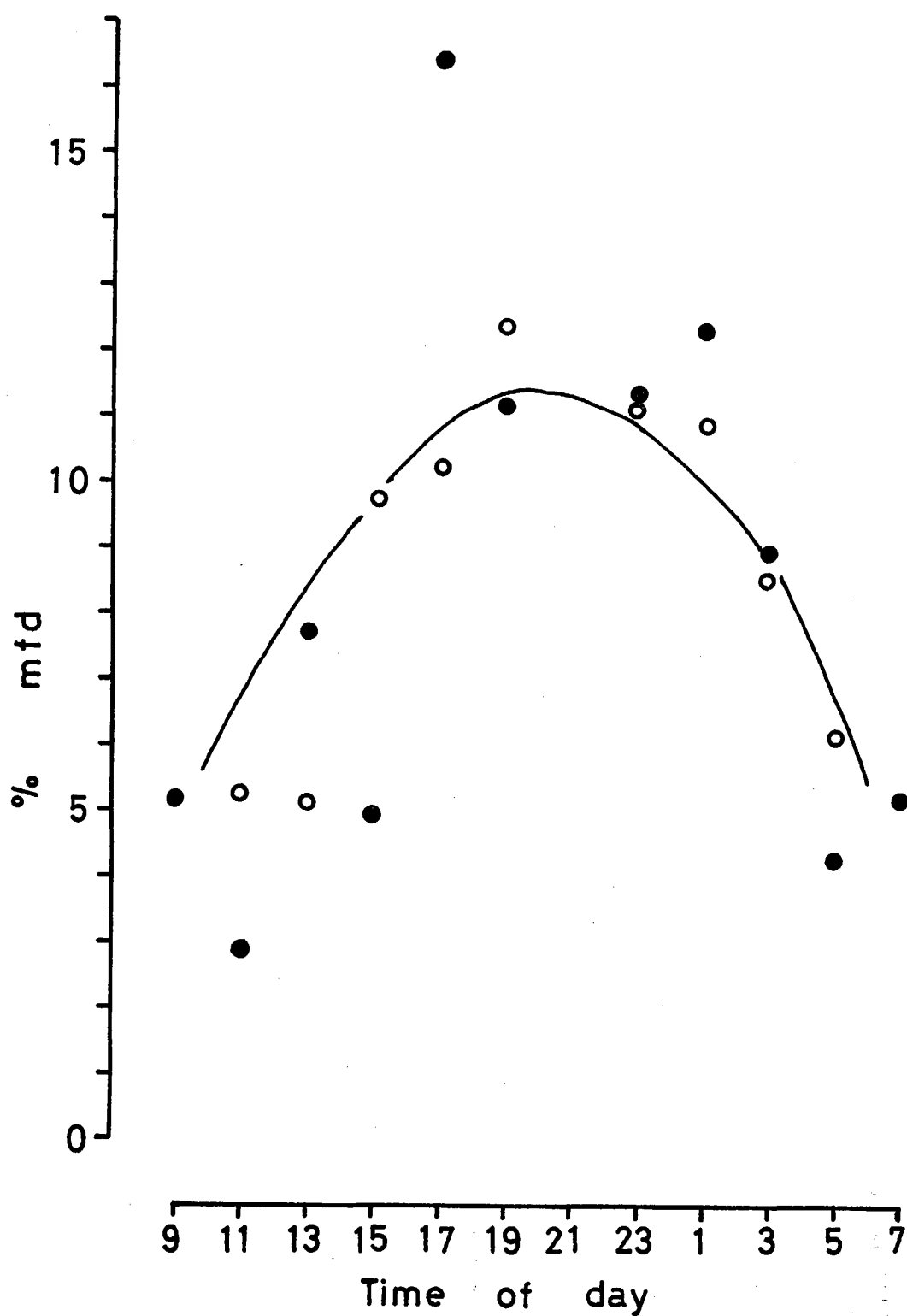


Figure 1 Microfilarial density of *O. volvulus* in 10 Guatemalan patient's skin, expressed as percentage per total mfd per day, in *Finca Nimaya*, Guatemala. ● mean % mfd; ○ triple-running average of mean % mfd.

filariae, quantitative skin biopsies were done at two endemic areas. In the first experiment, the biopsy was performed on four volunteers at intervals of two to four hours throughout the day, but the mfd varied with time and no clear-cut diurnal periodicity was recognized (Table 1). Table 2 and Fig. 1 give the results of second experiment by skin biopsies on 10 volunteers at the intervals of two hours in another endemic area. In this case, the mfds tended to show a higher rate during the period from evening to mid-night, 1700–0100 (Fig. 1), though individual subjects revealed a considerable fluctuation by the time of day in their mfds (Table 2). From the present two experiments, therefore, it is suggested that the mfds in Guatemalan onchocerciasis patient's skin may have a slight elevation from evening through mid-night, but not pronounced.

Table 2 Microfilarial density in patient's skin, expressed as percentage per total mfd, in an endemic area, *Finca Nimaya*, in Guatemala

Times	% mfd in each subject										Av. (%) ±s. d.	Gm**
	No. 1 (37.5*)	No. 2 (29.8)	No. 3 (23.4)	No. 4 (87.8)	No. 5 (66.2)	No. 6 (9.2)	No. 7 (155.1)	No. 8 (101.1)	No. 9 (40.9)	No. 10 (108.0)		
0900	8.8	2.0	1.7	8.2	7.9	6.4	0.3	1.4	6.6	8.1	5.1± 3.4	1.8
1100	5.6	0.3	5.6	6.2	1.8	0.0	0.3	8.4	0.5	0.0	2.9± 3.2	0.7
1300	16.3	1.3	33.8	2.3	0.6	3.2	3.2	3.2	7.3	6.1	7.7±10.2	2.2
1500	1.6	1.0	11.1	3.1	6.2	4.3	5.0	6.0	0.5	9.7	4.9± 3.6	1.8
1700	14.9	12.1	0.0	13.2	22.2	14.9	47.0	17.7	8.8	13.9	16.5±12.2	5.7
1900	17.1	16.4	3.8	16.6	23.1	2.1	5.4	8.7	5.4	12.7	11.1± 7.0	4.6
2100	7.2	13.1	11.5	5.0	0.0	9.6	17.2	10.9	6.1	17.2	9.8± 5.4	3.3
2300	10.7	27.5	4.7	3.3	11.3	14.9	0.1	12.8	24.4	3.8	11.4± 9.1	3.3
0100	5.9	4.0	10.7	13.8	11.0	26.6	4.3	14.8	25.2	6.6	12.3± 8.1	5.1
0300	5.3	11.1	0.0	16.5	13.7	16.0	0.9	7.4	4.4	14.2	9.0± 6.2	2.9
0500	4.0	9.4	0.8	2.3	1.1	0.0	6.0	7.6	10.8	0.7	4.3± 3.9	1.4
0700	2.7	1.7	16.2	9.6	1.1	2.1	10.4	1.1	0.0	6.9	5.2± 5.4	1.5

* Total mfd/mm²/day, ** Geometric mean.

Hourly microfilarial intake by S. ochraceum

Table 3 and Fig. 2 show the hourly microfilarial intake by the fly, *S. ochraceum*, which fed each microfilaria carrier. A total of 360 flies, 132 from No. 1, 73 from No. 2 and 155 from No. 3 volunteers, were examined for the microfilariae, performing a mass dissection of three to six flies per batch. The total mean numbers of microfilariae per fly in each human bait, thus obtained, was 94.5 in No. 1, 20.4 in No. 2 and 42.6 in No. 3 throughout the day. The hourly intake of microfilariae by *S. ochraceum* had two peaks, one in the early morning, 0700–1000 and the other in the mid- or late afternoon, 1400–1600. To know the variation of microfilarial numbers ingested by a fly, a part of the samples collected from each patient during the morning hours (0700–0800) was dissected individually. The mean number of microfilariae per fly was 15.1 ± 13.11 (n=15) in No. 1 (10.1 mfd), 3.5 ± 7.43 (n=12) in No. 2

Table 3 *O. volvulus* microfilarial intake by *S. ochraceum*, expressed as percentage per total mean recovery of microfilariae from flies in each volunteer, at various times of the day

Times	Volunteer No. and (mfd/mm ² /skin*)						Av. (%)± s. d.
	No. 1 (10.1 mfd)		No. 2 (13.9 mfd)		No. 3 (11.7 mfd)		
	No. fly exam.	% mf	No. fly exam.	% mf	No. fly exam.	% mf	
0600—	—	—	3	8.3	9	1.4	4.9±4.9
0700—	17	15.3	9	11.3	24	7.8	11.5±3.8
0800	13	9.2	11	14.2	20	18.3	13.9±4.6
0900—	8	3.3	4	1.5	19	8.0	4.3±3.4
1000—	12	4.8	12	11.3	18	13.1	9.7±4.4
1100	20	3.5	10	5.4	20	6.6	5.2±1.6
1200—	8	4.6	3	6.4	8	4.7	5.2±1.0
1300—	11	6.6	—	—	5	4.2	5.4±1.7
1400—	10	13.4	6	12.3	9	8.9	11.5±2.3
1500—	10	14.8	5	12.7	9	12.4	13.3±1.3
1600—	15	12.8	7	13.2	8	11.5	12.5±0.9
1700—	8	11.7	3	3.4	6	3.1	6.1±4.9

* Two skin snips each were taken from the volunteers at 15:30.

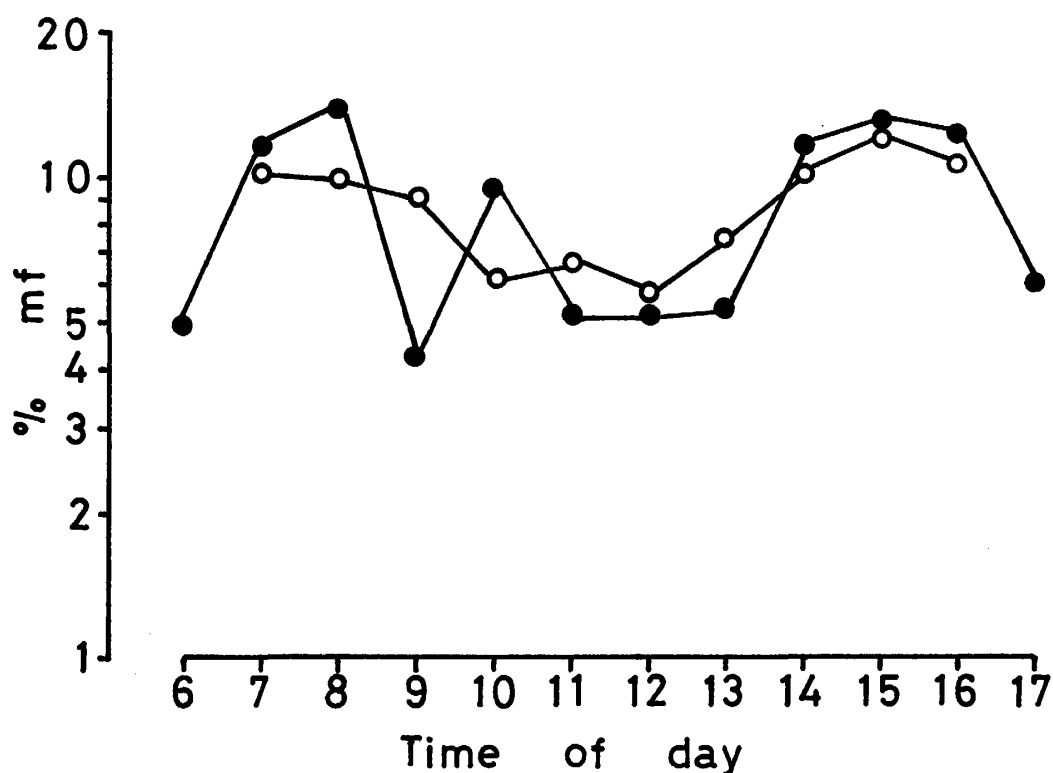


Figure 2 The intake of *O. volvulus* microfilariae by *S. ochraceum*, expressed as percentage per total recovery from flies in each human bait. ●—● mean % mf recovery; ○—○ triple-running average of mean % mf recovery.

(13.9 mfd) and 3.8 ± 4.32 ($n=15$) in No. 3 (11.7 mfd). Thus, the fly load indicated a great variation among the volunteers with a similar skin mfd. A similar observation on the low microfilarial intake by *S. ochraceum* was sometimes seen in the other field study.

Almost all of the microfilariae intaken by the fly were destroyed by the cibarial armature during ingestion; the damage reached 81.2% to 100% among those counted in the dissections of flies. Very low microfilarial intakes resulted in a high proportion of the destruction of microfilariae ingested.

DISCUSSION

It has been unknown whether the biting activity of black flies, *Simulium* spp., is influencing directly or indirectly on the *O. volvulus* microfilarial intakes. But there is a possibility that the microfilariae may be attracted by the vector, *S. ochraceum*, during ingestion (WHO, 1976; Hashiguchi *et al.*, 1981a). Therefore, the attracting condition of the black fly against skin microfilariae may be influenced by various factors, such as ecological and physiological ones, of the fly in various times of the day in endemic areas of the disease.

There were two peaks in the microfilarial intake, one in the early morning and the other in the late afternoon. However, this study is the first case reporting on the hourly intake of *O. volvulus* microfilariae by the vector, *S. ochraceum*. In order to have a more detailed and conclusive information, further such an examination should be done paying special attention to the parous ratios of *S. ochraceum* which showed distinct diurnal patterns (Collins *et al.*, 1981).

In the present study, the mfd of *O. volvulus* in 14 patient's skin was examined throughout the day in connection with microfilarial intakes by the fly, performing skin biopsies at 2 to 4 hour intervals. In patient's skin of one of the two experiments, the mfd rose slightly during the period from the late evening to mid-night indicating a considerable variation in individual volunteers. As to the mfd in skin snips taken at various times of the day, several studies are available in connection to the biting activity of the vector of Guatemalan onchocerciasis (Duke and Moore, 1974; Anderson *et al.*, 1975). Tada and Figueroa (1974), however, observed no diurnal periodicity in the skin microfilariae of *O. volvulus* in endemic areas of Guatemala. The discrepancy among investigators on the diurnal patterns of skin mfd may be due to the difference of sensitivities in skin biopsies. With the reason, it may be very difficult to know the true patterns of mfd in patient's skin by the biopsy techniques which are available in the moment. Collins *et al.* (1980), however, demonstrated that the sensitivity of the skin biopsy is enhanced by incubation for at least eight hours.

The result obtained suggests that there may be a diurnal pattern of microfilarial intakes of *O. volvulus* by *S. ochraceum*; the pattern, however, did not show any correlation with the present diurnal pattern of mfd in patient's skin. Further such an information on the hourly microfilarial intake would be necessary for a better understanding of the transmission mechanism of onchocerciasis. This study also showed that very low microfilarial intake resulted a high proportion of the destruction of microfilariae by the cibarial armature of *S. ochraceum*. The individual flies ingested such a low

density of microfilariae may be negligible in the light of their actual roles as a vector in the transmission of onchocerciasis.

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オンコセルカ症患者の仔虫密度とブユによる仔虫とり込み量の日周パターン¹

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グアテマラ共和国のオンコセルカ症流行地において、患者皮膚での仔虫密度とブユ *Simulium ochraceum* による仔虫とり込み量について、継時的観察を行った。患者4名について、2～4時間毎24時間、検皮法を実施した第1回目の実験では、仔虫密度に日周性を認めなかった。しかし、10名について2時間毎24時間の検皮では、午後5時から午前1時にかけて、仔虫密度の増加を認めた。一方、ブユにおける仔虫とり込み量は、午前と午後に高くなる傾向を示した。従って、今回の実験では、患者仔虫密度の日周性とブユによるとり込み量の間に、相関を見出すことはできなかった。仔虫とり込み量の“2つの山”は、ブユの吸血活動に何らかの形で関与する可能性が示唆された。*Onchocerca volvulus* 仔虫のブユによるとり込み量についての継時的な観察は、従来、皆無である。しかし、オンコセルカ症の伝搬機構を解明していく上で、同様な研究が今後必要であると思われる。

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