*Journal of Helminthology* (1981) 55, 95–100

# The mechanisms which affect the periodic cycle of Pacific *Wuchereria bancrofti* microfilariae

F. HAWKING,* TINOUSI JENNINGS, F. J. LOUIS and E. TUIRA

*LBJ Tropical Medical Center, Pago Pago, American Samoa and Institut de Recherches Médicales "Louis Malarde", Papeete, Tahiti*

## ABSTRACT

1. Investigations were made of the effect of various procedures in raising or lowering the microfilaria count of Pacific type *Wuchereria bancrofti* in the peripheral blood.
2. Raising the body temperature in the early morning was followed by a moderate fall in the counts. Breathing increased oxygen, or reduced oxygen (hypoxia) or increased carbon dioxide, or the ingestion of sodium bicarbonate produced no consistent and significant changes in the count. Ingestion of glucose (in one volunteer) was followed by a small rise in the count. Muscular exercise was followed by a fall in the count, which is interpreted as probably being a response to a lower concentration of oxygen in the venous blood returning to the lung.
3. It has not been possible to identify the physiological components of the circadian rhythm of the human body which entrain the cycle of these microfilariae. Attempts to obtain evidence incriminating the stimuli described above have been unsuccessful.

This paper reports investigations carried out in American Samoa and Tahiti on the mechanisms which control the 24-hour cycle of the microfilariae of Pacific type *Wuchereria bancrofti*. According to the observations of ROSEN (1955) these microfilariae show a peak at about 14.00–16.00 hours and a periodicity index of 20 to 25%

$$\left(\text{i.e.}\frac{\text{standard deviation}}{\text{mean}} \times 100\right)$$

but this was not tested during the present work.

## METHODS

The investigations were made during February 1979 at the LBJ Tropical Medical Center, American Samoa, and at the Institut de Recherches Médicales, Tahiti. The microfilaria carriers who kindly co-operated were two adult Samoans and four adult Tahitians (one female) who were confirmed as being healthy in all respects apart from their microfilariae. The numbers of microfilariae in the blood were counted by taking four measured volumes of 20 $mm^3$ finger blood, drying them as thick films, and staining with Giemsa in the usual way. The body temperature was recorded by an electric thermometer reading to 0·1°C with the probe placed in the mouth. Oxygen gas was given through a mask lightly attached to the face. Other gas mixtures were administered through a mask held in the operator's hand near the face and continually adjusted according to circumstances. Exercise was effected by the volunteer stepping up and down off a stool 35 cm high about 12 to 20 times a minute. The exercise produced mild sweating and hypernoea but never approached exhaustion.

* Address: 14 Hillside Road, St. Albans, Herts, England.

## RESULTS

*Effect of body temperature*

To investigate the effect of changing the body temperature, two Samoan volunteers were taken in the early morning when their temperature and the microfilaria counts were both low, and they were asked to sit in warm baths so that the body temperature was raised.

In the first man, the microfilaria count was 501 mf/40 $mm^3$ at 16.00 which was presumably near the maximum and it was still 433 at 23.50.

| Time | Mf count/ 40 $mm^3$ | Percentage of initial count | Body temperature °C |
|---|---|---|---|
| 03.00 | 273 | 100 | 36·1 |
| 03.10 | the man began to sit in a warm bath | | |
| 03.40 | 230 | 84 | 38·3 |
| 04.10 | 256 | 93 | 38·4 |
| 05.10 | 212 | 77 | 38·5 |
| 05.15 | the man got out of the bath | | |
| 05.50 (in bed) | 205 | 75 | 37·4 |
| 07.05 (in bed) | 307 | 112 | 36·8 |

*Summary*: During the period when the body temperature was raised, the microfilaria count tended to *fall* and after two hours it was approximately 77% of the initial count. Throughout the experiment the changes seen in the count might have been due to the normal 24 hour cycle.

In the second man, the peak microfilaria count in the afternoon (14.30) was 60 mf/80 $mm^3$

| Time | Mf count/ 80 $mm^3$ | Percentage of initial count | Body temperature °C |
|---|---|---|---|
| 02.15 | 43 | 100 | 36·4 |
| 02.25 | the man began to sit in the warm bath | | |
| 02.55 | 45 | 105 | 38·2 |
| 03.25 | 39 | 91 | 38·8 |
| 03.55 | 33 | 77 | 38·6 |
| 04.30 | 33 | 77 | 38·5 |
| 04.35 | the man got out of the bath | | |
| 04.55 (in bed) | 37 | 86 | 37·0 |

*Summary*: During the period when the body temperature was raised, the microfilaria count tended to *fall* (perhaps due to the 24 hour cycle); it certainly was *not* elevated.

*Other stimuli*

Various other investigations were made in series during the daytime on the same subjects in Samoa (S) and Tahiti (T). In a typical case, oxygen was administered for 20 min; then an interval of 45 min; then carbon dioxide for 10 min; then an interval of 45 min; then exercise for 10 to 15 min; an interval of 45 min; hypoxia 5 to 10 min; an interval of 45 min as illustrated in Fig. 1. Blood for microfilariae was taken before and after each stimulus and also during the middle of the longer stimuli. The microfilaria counts after each stimulus are expressed as percentages of the mean of all the 10 to 13 counts (including the experimental ones) during that series of investigations. These observations were made between 14.00 and 17.00 when the count is high. The results are shown in Table I.

*Oxygen* was administered to five volunteers. In three there were no changes in the microfilaria count sufficiently large or sustained to be significant in the judgement of

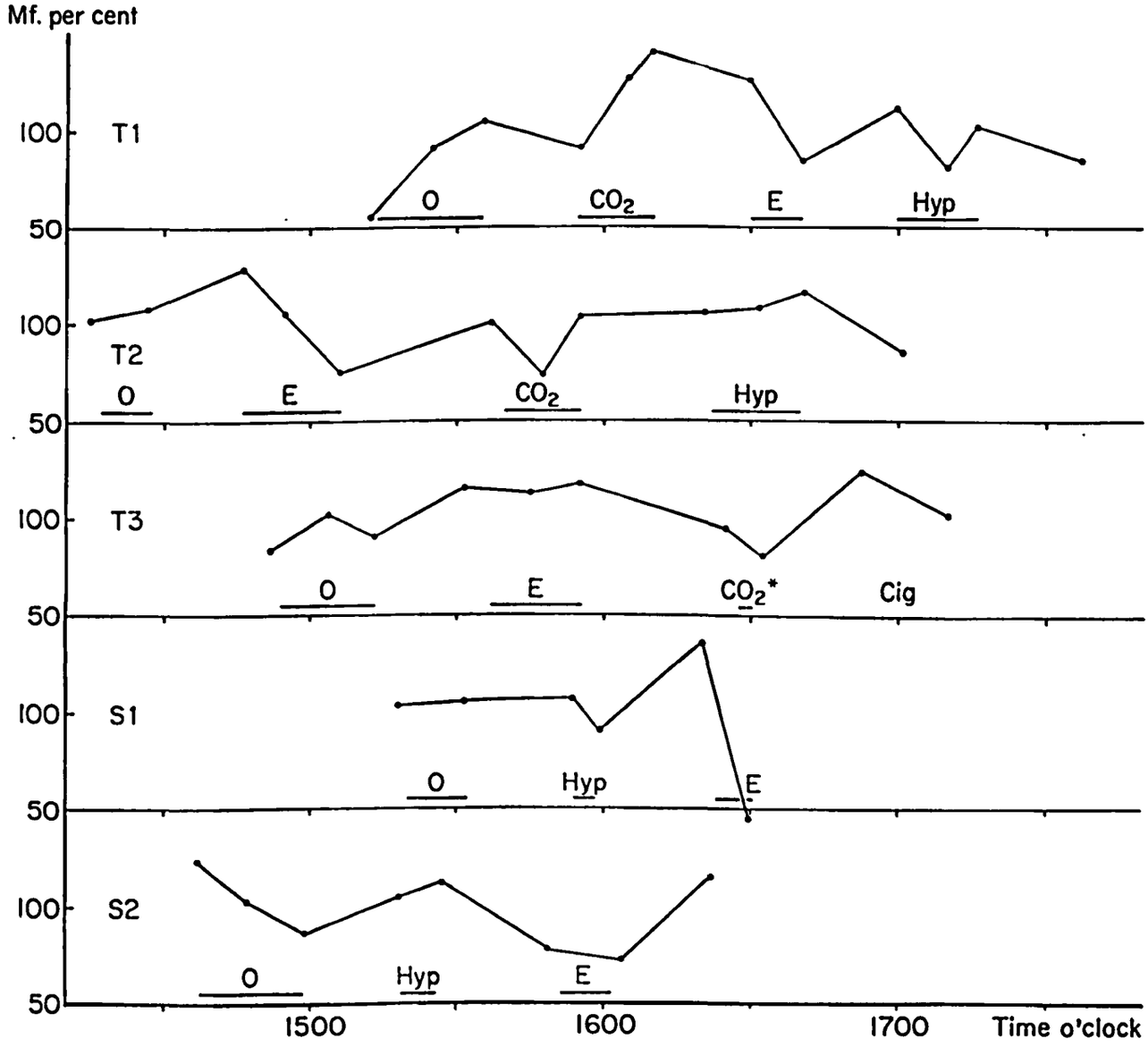


FIG. 1. To show the changes in the microfilaria count during the application of various stimuli to 3 Tahitian and 2 Samoan volunteers. The microfilaria counts are given as percentages of the mean of all the counts during the experiment. The application of stimuli is shown by the short thick horizontal lines, O = oxygen; $CO_2$ = carbon dioxide; E = exercise; Hyp = hypoxia, *indicates alarm and distress, Cig = cigarettes (see text).

TABLE 1

The changes produced by various experimental procedures on the numbers of Pacific *W. bancrofti* microfilariae in the blood

| Procedure | Volunteer | Microfilariae Mean count per 80 × mm³ | Microfilaria counts as percentage of mean: Initial count | After procedure[1] | Percentage change[3] | Later count[2] |
|---|---|---|---|---|---|---|
| Oxygen | T1 | 242·4 | 55·2 | 92·4(+37·2)→104·4(+49·2) | +90 | 99(−5·4) |
| | T2 | 200·0 | 101·5 | 108·0(+6·5) | +6·4 | 129(+21) |
| | T3 | 187·2 | 83·5 | 101·5(+18·0)→90·3(+6·8) | +8·2 | 116(+25·7) |
| | S1 | 966·0 | 104·0 | 105·3(1·3) | +1·2 | 106·8(+1·5) |
| | S2 | 48·8 | 123·0 | 102·0(−21)→86·0(−37) | −37 | 104(+18) |
| Hypoxia | T1 | 242·4 | 111·0 | 79·4(−30·6)→103·0(−8·0) | −7·2 | 85·8(−17) |
| | T2 | 200·0 | 105·0 | 108·5(+3·5)→115·5(+10·5) | +10 | 86·5(−29) |
| $CO_2$ | T1 | 242·4 | 99·0 | 127·0(+28)→140·5(+41·5) | +42 | 126(−14·5) |
| | T2 | 200·0 | 99·5 | 73·5(−26)→104·0(+4·5) | +4·5 | 105(+1) |
| Exercise | T1 | 242·4 | 126·0 | 103·0(−23) | −18 | 135(+32) |
| | T2 | 200·0 | 129·0 | 104·0(−25)→73·0(−56) | −43 | 99·5(+26·5) |
| | T3 | 187·2 | 116·0 | 102·0(−14)→117·7(+1·7) | +1·5 | 94·0(−24) |
| | S1 | 966·0 | 136·0 | 45·0(−91) | −67 | |
| | S2 | 48·8 | 78·0 | 72·0(−6) | −7·8 | 118·0(+46) |

[1] The first count was taken after the procedure had been applied for 7–10 min and the second after the procedure had lasted for 15–20 min. The assessment of effect was taken after the second count when available. The figures in parentheses indicate the change from the initial count.

[2] The later count was taken after the volunteer had rested for approx. 45 min. The figure in parentheses indicates the change from the count at the end of the procedure.

3 Change as percentage of initial count.

the authors. In one volunteer (in whom the initial count was only 55% of the mean) there was a rise of 49%; and in one volunteer (in whom the initial count was 23% above the mean) there was a fall of 37%. In both these last two cases the count tended to return to its mean value. These results are in general agreement with those previously obtained in Fiji (EDESON, HAWKING & SYMES, 1957). On that occasion four volunteers were given oxygen during the daytime. The counts fluctuated considerably and although the counts tended to *rise*, it was considered that too much significance must not be attached to this increase. It is probable that increased oxygen has no consistent effect on the level of Pacific microfilariae in the peripheral blood; certainly it does not produce the abrupt *fall* which it does with the microfilariae of nocturnal *W. bancrofti* (see HAWKING, 1967).

*Hypoxia*: Two volunteers breathed atmospheres with a reduced content of oxygen. In one volunteer after 10 min there was a fall in the microfilaria count but after a total of 16 min hypoxia the fall was only 8%. In the other volunteer after 20 min of hypoxia there was a small rise of 10%. These investigations showed no significant effect after hypoxia. In three volunteers studied in Fiji in 1955 there was a tendency for the count to *rise* during hypoxia.

*Carbon dioxide*: Two volunteers breathed atmospheres with increased carbon dioxide sufficient to produce hypernoea. In one man the microfilaria count was raised by 41% of the mean, but in the other there was no significant change. In three volunteers




https://doi.org/10.1017/S0022149X00025554

studied in Fiji in 1955, the increase of carbon dioxide produced no consistent increase in the count.

*Exercise* was tested on five volunteers. In three the microfilaria count decreased by 23 to 91% of the mean, and in two there was no significant change. In three subjects in Fiji in 1955, there was always a decrease in the count by an average of 62% (see Discussion).

*Sodium bicarbonate*: Starting about 02.00, sodium bicarbonate 10 g dissolved in a little water was given by mouth three times at 14-min intervals to two men (T1 and T4) and to one of these men (T1) on a different night it was given twice. The men had not eaten recently. The salt was well tolerated without any signs of nausea. During the period up to 15 min after the last dose, the microfilaria count fell gradually in two of the volunteers (by 12% of its initial value in T4 and by 35% in T1) but in one volunteer (T1, two doses) it rose by 23%. The counts were then followed at 15-min intervals until one hour later but they showed no consistent changes. In normal physiology the body tends to become more alkaline by day (and the count rises to its maximum in the afternoon) and to become more acid by night (when the count falls). It is not known how far the hydrogen reaction of the blood was actually altered by this administration of sodium bicarbonate but these observations gave no evidence for a correlation between the microfilaria count and the alkalinity of the body.

*Blood sugar*: One volunteer (T4) swallowed 100 g glucose in a little water at 02.00. 15 min later the microfilaria count had risen to 131% of its initial value and then it fell progressively to 65% at two hours after the glucose (see Discussion).

*Other observations*: One female volunteer (T3) became temporarily disturbed and alarmed by an attempt to administer carbon dioxide from a noisy cylinder; during this time the microfilaria count fell to 85% of its previous value. She sat for half an hour to rest and the count rose to 132%. After this she smoked two cigarettes and the count became 110%. The significance of these changes is uncertain but they are recorded as being of possible interest, suggesting that microfilaria count is not greatly changed by alarm or cigarettes. Perhaps nervous alarm causes the count to fall.

## DISCUSSION

Many studies have been made in the past on the periodicity of microfilariae and the conclusions have been sumarized in the reviews by HAWKING (1967, 1975). Briefly, it is considered that microfilariae in general tend to accumulate in the arterioles of the lungs, which is the point in the circulation where the oxygen tension is lowest just before the blood enters the pulmonary capillaries and takes up more oxygen. It is believed that this accumulation is due to the microfilariae making active responses to avoid entering the zone of higher oxygen tension, which may be called the 'oxygen barrier'. The circadian rhythms of the host produce 24-hour variations in this barrier (or in the sensitivity of the microfilariae to it) and consequently they produce 24-hour variations in the number of microfilariae passing through it and appearing in the peripheral blood. Different species of microfilariae respond to different host rhythms. Thus with noctural *W. bancrofti*, the microfilariae are very sensitive to changes in the oxygen tension, which is lower in the pulmonary capillaries at night so that the microfilariae pass through it more readily and the microfilarial count in the peripheral blood goes up. With *Loa loa* the sensitivity of the microfilariae is modified by changes of body

temperature; when the body temperature rises by day, the sensitivity becomes less and so the microfilariae pass through the barrier more readily and the count rises in the daytime.

The microfilariae of Pacific *W. bancrofti* show a 24-hour cycle of moderate amplitude with a maximum at 14.00 to 16.00. According to the above hypotheses, this variation should be entrained by some circadian rhythm of the host which is maximal (or minimal) at 14.00 to 16.00 and conversely at 02.00 to 04.00. The observations recorded in this paper may be considered from this point of view. It was found above that raising the body temperature at night caused a *fall* in the microfilaria count rather than a rise. According to a personal communication, kindly sent some years ago without details, Dr. J. U. Mataika of Fiji has also observed no clear response in the microfilaria count when the body temperature was raised by night. Consequently it is unlikely that the microfilaria cycle is entrained by changes in temperature (as is the case with *L. loa*).

Judging by the results described above and those obtained in Fiji by EDESON, HAWKING & SYMES (1957) there is no evidence that the microfilarial cycle of Pacific *W. bancrofti* depends upon circadian changes in the oxygen tension, carbon dioxide tension, or alkalinity of the blood. The level of blood sugar is uncertain; the figures obtained above are suggestive but it is only a single experiment. The stimulus which produced the most consistent response (a fall) in the microfilarial count was muscular exercise; but this would not explain the diurnal periodicity of these microfilariae since muscular exercise is minimal in the early morning when the count is at its lowest. It must regretfully be concluded that the body cycles which entrain the cycle of Pacific microfilariae are still unidentified. Attempts to obtain evidence incriminating certain cycles (body temperature, oxygen changes etc.) have been unsuccessful.

ACKNOWLEDGEMENTS

Grateful acknowledgements for facilities and assistance are due to Dr. Cheeseman, Dr. M. Beck and their colleagues in Samoa and to Dr. J. Laigret and his colleagues in Tahiti.

## REFERENCES

EDESON, J. F. B., HAWKING, F. & SYMES, C. B. (1957) The periodicity of microfilariae. VI. The response of microfilariae of *Wuchereria malayi* and *W. bancrofti*, Pacific type, to various stimuli. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **51**, 359–365.

HAWKING, F. (1967) The 24 hour periodicity of microfilariae: biological mechanisms responsible for its production and control. *Proceedings of the Royal Society B*, **169**, 59–76.

HAWKING, F. (1975) Circadian and other rhythms of parasites. *Advances in Parasitology*, **12**, 123–182.

ROSEN, L. (1955) Observations on the epidemiology of human filariasis in French Oceania. *American Journal of Hygiene*, **61**, 219–248.

*Accepted 15 November 1980.*