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LIGHT-ON EFFECTS AND THE QUESTION OF BIMODALITY IN THE CIRCADIAN FLIGHT ACTIVITY OF THE MOSQUITO *ANOPHELES GAMBIAE*

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INTRODUCTION

In an alternating 12 h light: 12 h dark regime (LD 12:12), in the laboratory, female *Anopheles* (*Cellia*) *gambiae* Giles have peaks of non-specific flight activity at light-off and light-on; they are moderately active during the remainder of the dark period (Jones, Hill & Hope, 1967). Previously (Jones, Cubbin & Marsh, 1972), we have concentrated on the 'light-off' peak as it appears to be the best indicator of the phase of the circadian rhythm. In this paper we discuss the evidence that the activity pattern is bimodal, the sharp initial peak being followed by secondary activity with a less well defined mode. In the wild, this secondary activity may provide a permissive framework for host-seeking and biting. We also describe the effect, on the pattern, of changing the time of light-on (light-off constant) and confirm that the light-on activity is a reaction to the sudden onset of light.

METHOD

The activity of individual sugar-fed females was recorded using the flight sound as an indicator. The basic technique was that of Jones *et al.* (1972).

The mosquitoes came from the Lagos and Ibadan cultures of species A which were used in previous experiments. The Ibadan culture was used only for the 'dawn and dusk' experiments.

The light intensity was approximately 70 lux produced by two 15 W tungsten-filament bulbs. Light-on and light-off were abrupt except in the 'dawn and dusk' experiments when the change was made with a modification of the programming device described by Belton, Kemster & Nair (1967). The programming apparatus was used to control two 15 W tungsten-filament bulbs instead of fluorescent tubes so that there should be a smooth transition between light and complete darkness. The change from light to dark and vice versa took 30–40 min. In Figs. 1 and 2 the activity is grouped in periods of 1 h as this reveals more clearly the importance of the long-lasting moderate activity. Half-hourly periods are used in the other figures as this gives more information about the timing of the major peaks of intense activity.

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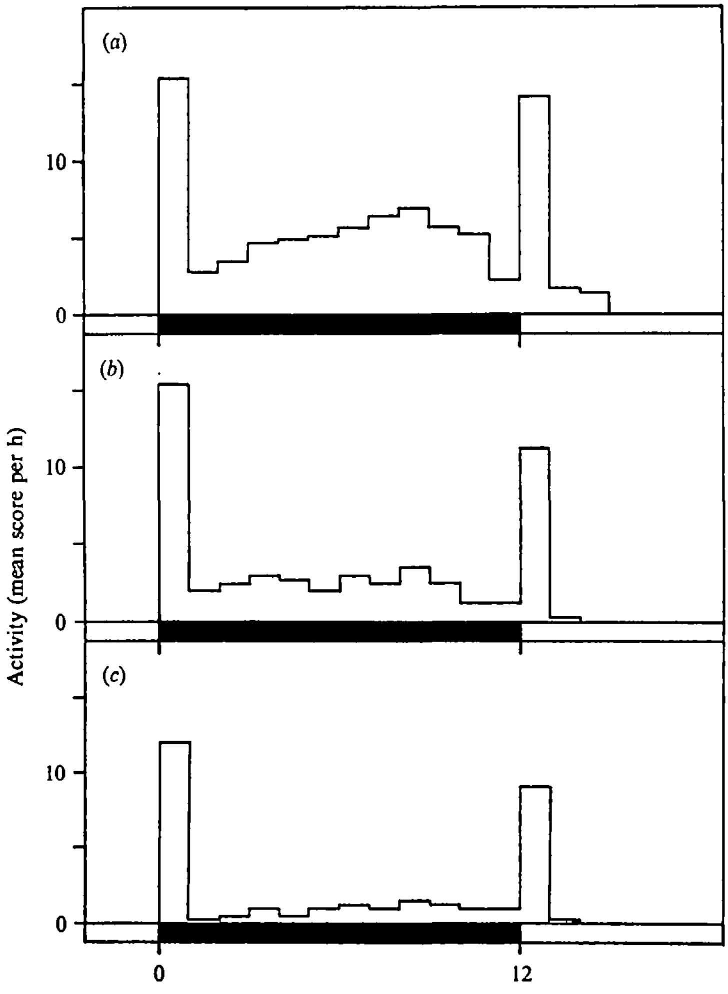

Fig. 1. Activity in LD 12:12 (Lagos females). Activity level (units per 24 h): (*a*) > 70 ($n = 22$), (*b*) 40–69 ($n = 24$), (*c*) < 40 ($n = 43$).

RESULTS

LD 12:12

Effect of activity level

Fig. 1 shows the mean pattern of activity in LD 12:12 at three different activity levels, measured by the number of units per 24 h (units = min with activity); Table 1 analyses the distribution of activity in these three histograms. All show the characteristic peaks following light-off and light-on. When the activity level is low, these peaks dominate the pattern and some individuals may show no other activity. Despite this, even in Fig. 1 (*c*), the moderate activity during the rest of the dark period still accounts for 30% of the total; in Fig. 1 (*a*) this proportion is over 60%. This 'secondary' activity is fairly diffuse, but may reach its maximum 6–10 h after light-off.

Table 1

Mean activity level (units/24 h)	% of total activity: In 1 h after light-off	During rest of dark period	Following light-on
86	18	62	20
53	29	49	22
32	38	32	30

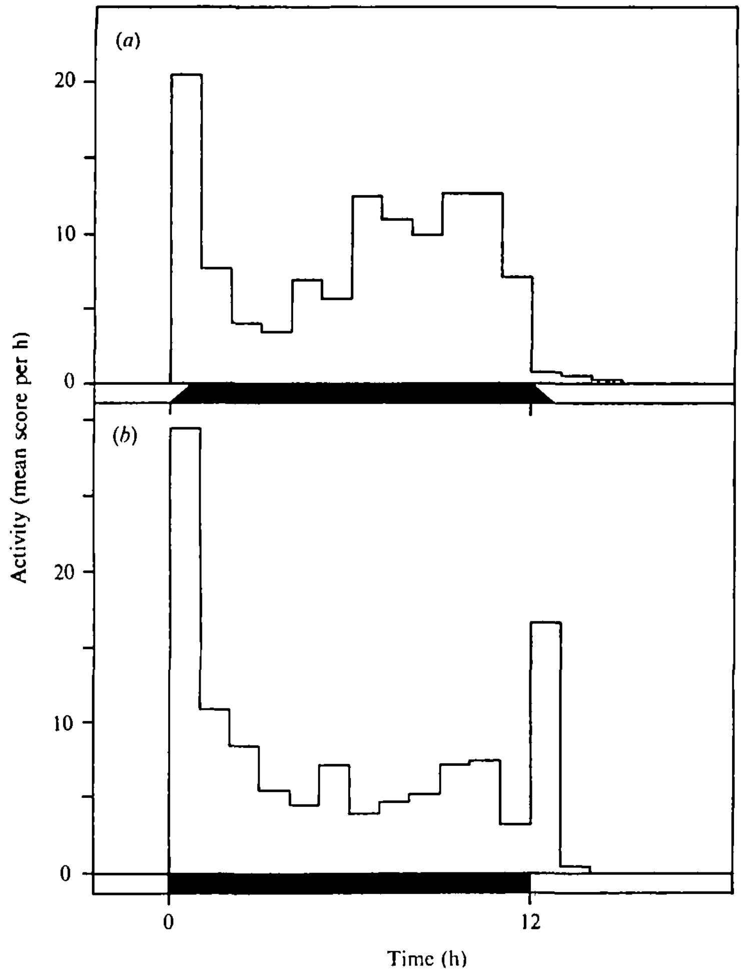

Fig. 2. Activity in LD 12:12 (Ibadan females). (*a*) With artificial 'dawn and dusk'. (*b*) With abrupt light-off and light-on.

Artificial 'dawn and dusk'

Fig. 2(*a*) shows the mean activity of 12 females from the Ibadan culture during 3 cycles of the regime. In this experiment the mean activity was 114 units per 24 h. For comparison, Fig. 2(*b*) shows the mean activity of the most active Ibadan replicates (activity > 100 units per 24 h) in a conventional regime with an abrupt light-on and light-off (mean of 19 individual cycles, mean activity = 115 units per 24 h).

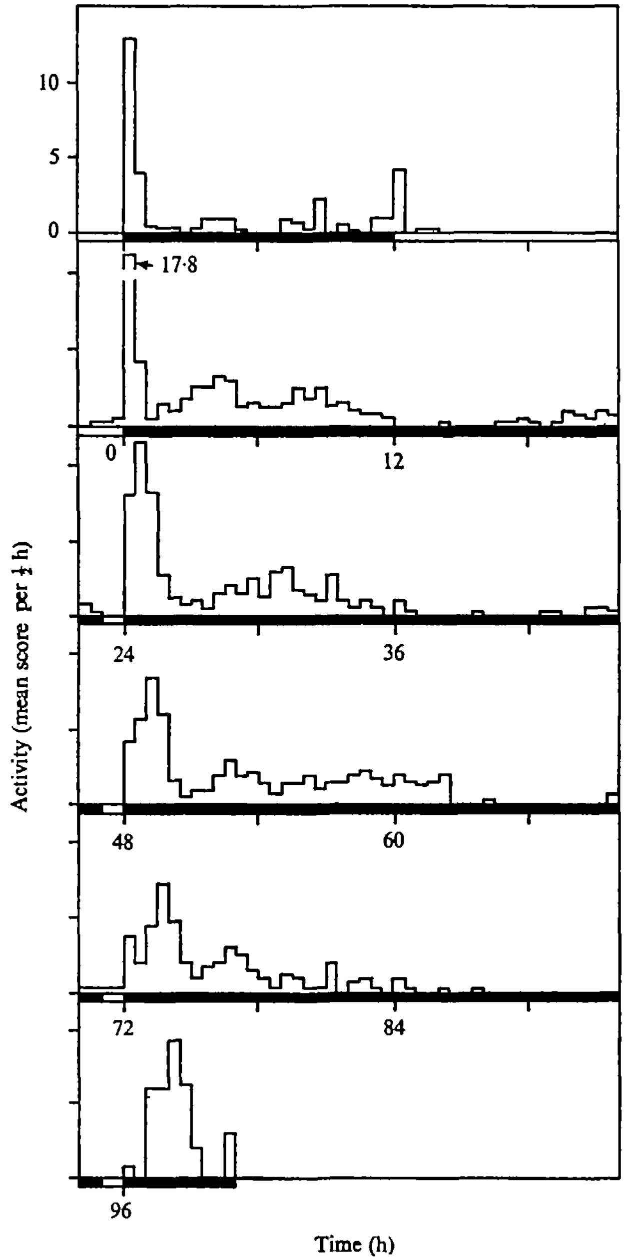

Fig. 3. LD 12:12 to LD 1:23 (mean activity of 16 females). On the abscissa, 0 = the last light-off of the old regime.

In the 'dawn and dusk' regime there is still a peak at light-off (experiments to measure the threshold will be reported later), but the light-on peak disappears. Preliminary work with the Lagos culture carried out in this laboratory, as an undergraduate project, by Mr S. R. Webb, gave similar results.

In Fig. 2(*a*) the secondary activity is very apparent, probably because of the high level of activity, but it has almost finished before 'dawn'.

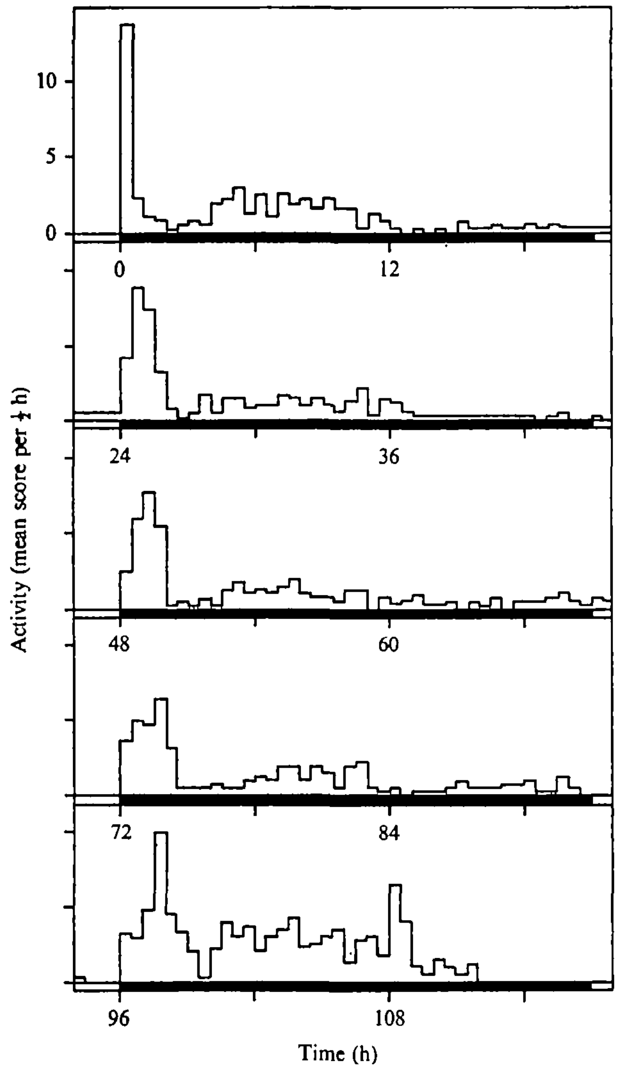

Fig. 4. LD 12:12 to LD 3:21 (n = 11). The final cycle in the rearing regime was not recorded.

Changing the time of light-on (Lagos culture)

Late light-on, short light period

When the regime is changed to LD 1:23 (Fig. 3) or LD 3:21 (Fig. 4) the first 'light-off' peak of the new regime appears about ½ h later than normal; in subsequent cycles the peak is progressively later, until in the fourth cycle it is 1½–2 h after light-off.

In an LD 6:18 regime (Fig. 5) the 'light-off' peak remains in the first ½ h after light-off although some individuals have peaks up to 1½ h later.

Figs. 3–5 show pronounced secondary activity which appears to finish about 12 h after the initial peak. The light-on peak disappears in the new regimes.

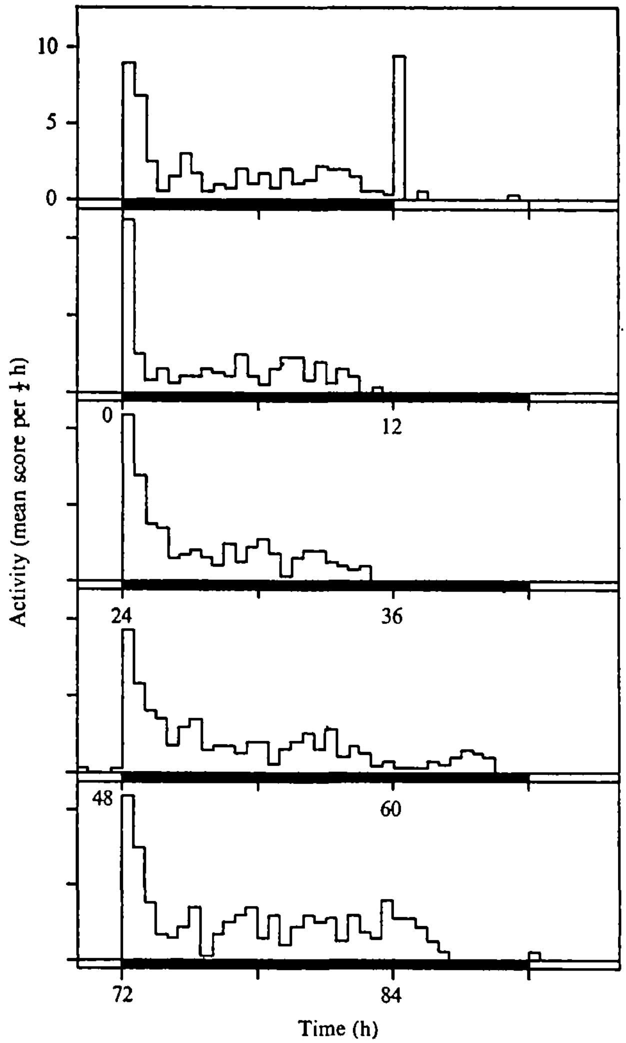

Fig. 5. LD 12:12 to LD 6:18 ($n = 14$).

Early light-on, short dark period

In these regimes (Figs. 6–8) the initial peak is at the normal time, just after light-off. In the LD 18:6 regime (Fig. 6) there is a light-on peak at the new light-on time and a persistent small peak about 3 h after light-on (more than half the individuals show some activity at this time). This is similar to the small burst of activity observed by Jones *et al.* (1967) in the first cycle after an LD 12:12 regime had been advanced by 6 h.

In the first cycle of an LD 21:3 regime (Fig. 7) there is a light-on response and a small peak of activity about 5 h after light-on. In the next cycle both these peaks disappear; possibly this is associated with the decrease in the mean activity from its basal level of approximately 50 units per 24 h to 28 units per 24 h. The activity level increases again during the following cycles until it reaches a level of 60 units in the

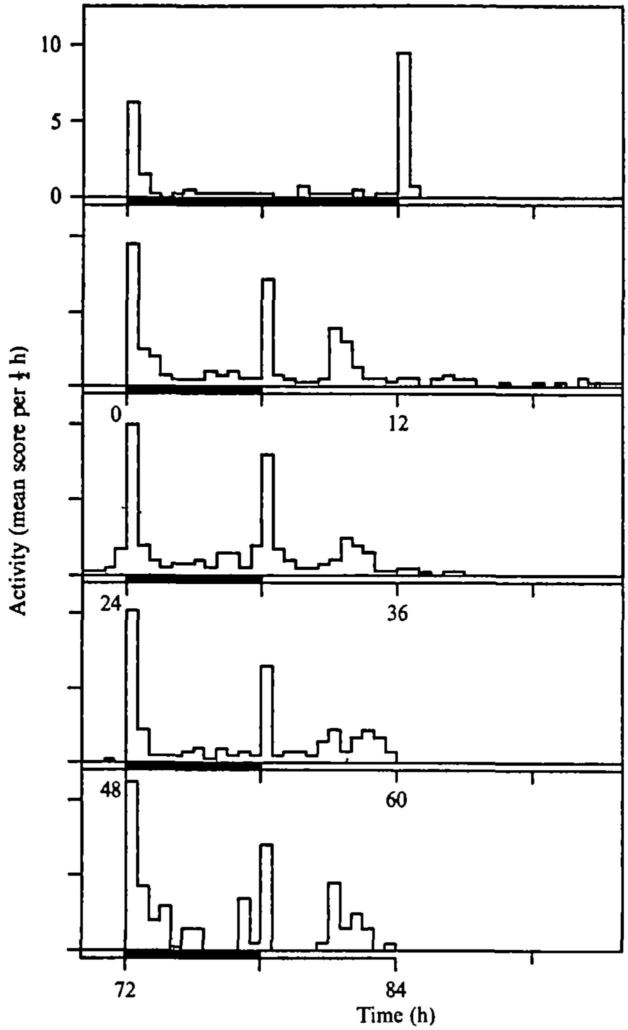

Fig. 6. LD 12:12 to LD 18:6 (n = 17).

fifth cycle. The light-on peak reappears, but there is little evidence of the other peak apart from some activity following light-on in the fifth cycle.

In the first cycle of the LD 23:1 regime (Fig. 8) the activity level is 45 units per 24 h and some activity 'overflows' into the light period. In the second cycle the activity level has decreased to 24 units and there is little activity after light-on. During the next three cycles some activity reappears in the ½ h following light-on but there is little change in the mean activity level.

DISCUSSION

Timing of the initial activity peak

After a change to LD 1:23 or 3:21 (Figs. 3, 4) the period of the cycle is approximately 24½ h for at least the first 4–5 cycles of the new regime. Thus the peaks appear progressively later with respect to light-off. The phase-response curve (Jones *et al.*

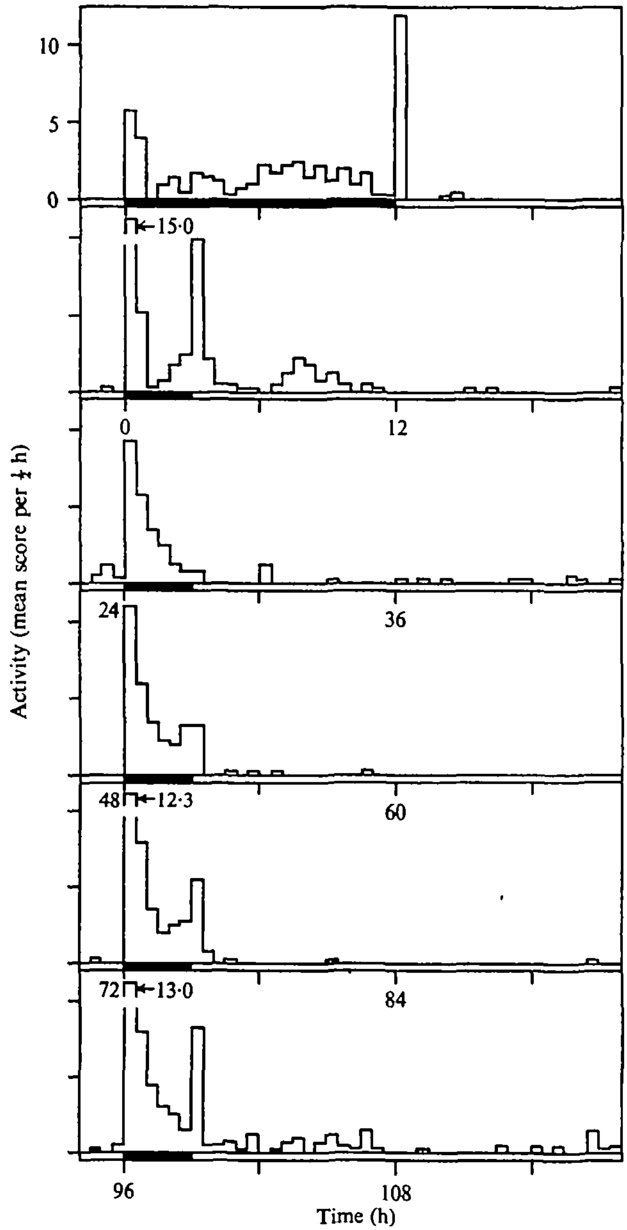

Fig. 7. LD 12:12 to LD 21:3 (n = 10).

1972) indicates that the light is acting at a time in the cycle when its net effect is to increase the period or delay the phase. It is to be expected (Pittendrigh, 1965) that as the light comes earlier in each cycle it will eventually reach a position where its net effect is to maintain the period at 24 h and thus there will be a new equilibrium position for the peak.

As L increases, the net effect seems to be a compromise between the tendency of the first light to shorten the period of the cycle and the last light to lengthen it.

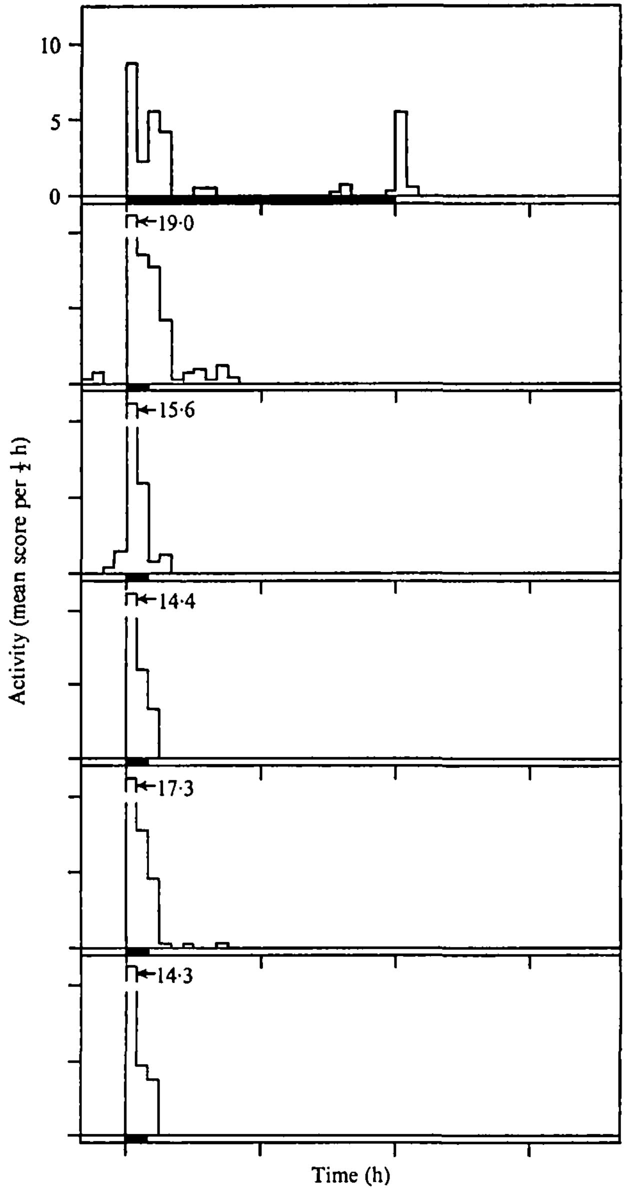

Fig. 8. LD 12:12 to LD 23:1 (n = 5).

In LD 6:18 the period of the mean activity cycle remains at 24 h although it may be longer in some individuals. When L ⩾ 12 h the timing of the activity peak appears to be determined mainly by the inhibitory effect of the light; thus the regime imposes a period of 24 h on the activity cycle.

Light-on activity

A burst of activity only follows light-on if it comes during the period of about 12 h following the main activity peak. In LD 21:3 and 23:1 light-on activity disappears after one cycle of the new regime, but reappears in later cycles. This may be linked with the initial reduction of activity during these regimes.

When the change of light-intensity is made slowly (artificial dawn) the light-on activity disappears. Thus it appears to be a 'startle' reaction to the abrupt switching-on of the light as suggested by Jones, Ford & Gillett (1966).

Secondary activity

If D $\geqslant$ 12 h the insect remains moderately active for about 10–12 h after the initial activity peak. At high levels of activity this secondary activity accounts for a considerable proportion (*c.* 60 %) of the total activity and is more pronounced during the period 4–10 h after the main peak. This activity appears to be related to the minor bursts of activity observed during the light period when D = 6 or D = 3. In LD 18:6 this 'peak' is approximately 9 h after light-off, and in LD 21:3, 8 h after light-off. These 'peaks' appear to depend on the activity level and this may be reduced in regimes with a high LD ratio. Jones *et al.* (1967) have suggested that such peaks may be the result of the interaction of a persistent excitatory hormone and the inhibitory effect of light.

Secondary peaks appear to be quite common in circadian activity cycles. Chiba (1964) found that the mosquito *Culex pipiens pallens* showed two peaks of activity per 24 h even in constant dark and, as a result of his work with finches, Aschoff (1966) has concluded that the basic two-peak pattern of locomotor activity is a persistent property of the circadian oscillating system. Roberts (1960) observed a secondary peak of activity in the cockroach (*Leucophaea*) and thought that, as it came at the normal time of dawn in the natural environment, it might prepare the insect for the onset of light and thus make it seek a dark resting place. Taylor (1969) observed secondary peaks in a number of mosquitoes and concluded that, in dark-active insects, they reinforce the dawn peak in light-regimes normally encountered by the species. In *A. gambiae* this 'secondary' activity is more easily identified with the normal biting activity in the latter half of the night (Gillies & De Meillon, 1968), but it is interesting that the mosquitoes only give a light-on response during the half of the cycle in which secondary activity can be expected. The light-on response appears to be 'primed' by the rhythm.

A bimodal pattern of activity does not necessarily indicate that the underlying rhythm has two peaks. The rhythm may inhibit activity during approximately half the cycle, possibly through some threshold effect. When the rhythm first permits activity, the release from inhibition might account for the initial peak; a rtough could follow this because of the exhaustion of necessary metabolites. Throughout the 'permissive' period activity may occur 'spontaneously'; various factors under the control of the rhythm may increase the tendency for spontaneous activity during this half of the cycle. It is interesting that, after the main peak, the flight activity of individual *A. gambiae* females may occur in discrete bursts at fairly regular intervals (Jones *et al.* 1967), possibly each time the threshold is reached. Brady (1970) observed similar regular bursts of 'spontaneous' activity in tsetse flies and noted that the duration of the bursts was comparable with the known course of amino acid consumption during forced flight. Further work on this aspect may lead to a better understanding of the mechanisms involved.

SUMMARY

1. The pattern of non-specific flight activity in *Anopheles gambiae* females appears to be bimodal, with an initial peak followed by about 12 h of secondary activity, with a maximum 6–10 h after the main peak.

2. The secondary activity is more apparent when the activity level is high, and can account for over 60% of the total activity. When D < 12 h activity is curtailed by the inhibitory effect of light, but there may be a minor peak in the light period, 8–9 h after light-off.

3. The main peak normally follows light-off, but is delayed in LD 3:21 and 1:23.

4. There may also be a peak at light-on if it is abrupt and occurs during the active half of the cycle. The peak is not observed if the light-intensity is changed slowly.

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