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Tick-Host Relationships 1. The Existence of a Circadian Rhythm of "Drop-off" of Engorged Ticks from their Hosts *

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Increasing number of physiological and behavioural activities of living organisms are shown to manifest a diurnal pattern characterized by a daily repeated rhythmic variation.

In some cases such rhythmically varying biological activities have been found to persist under constant conditions in the laboratory, thus implementing environmental factors, such as light, temperature etc. not to be the main factors involved.

The term "twenty four hours rhythm", applied to this phenomenon in the field has been abolished due to the fact that under laboratory conditions such rhythmic cycles might last anything but 24 hours. Consequently the term "circadian rhythm" has been proposed to describe a rhythmically changing biological activity "that will persist for at least 2-3 days in a constant environment, being little affected by temperature within normal biological limits, and that the timing of the peaks or phases of the rhythm in relation to solar time is originally determined by an environmental change such as a variation in light intensity or temperature" (HARKER, 1961).

The exact nature of the mechanism of the circadian rhythm is not known. It appears, however, that its pattern is determined by an "inner-clock", dependent upon endogenous physiological processes and brought about by a neuro-secretory system while "the phases or peaks of any such diurnal rhythm may be set in relation to solar time by a variety of environmental factors" (HARKER, 1961). Environmental conditions might determine the period length of the diurnal cycle within the limits offered by the genetic constitution of the individual.

The existence of diurnal rhythms under natural conditions has been clearly shown in various biological activities such as flight times, biting and oviposition cycles.

It has been described for the process of oviposition in two species of *Crambus* (CRAWFORD, 1966, 1967), for spermatogenesis in the house finch *Carpodacus mexicanus* (HAMNER, 1966), biological activity of *Periplaneta americana* (HARKER, 1960; ROBERTS, 1966). Circadian patterns have been similarly described for the appearance of amino acids in the blood of adult human beings (FEIGIN et al., 1967) and for the control of chitin macromolecular orientation by the cells secreting and organizing the endocuticle in insects (NEVILLE, 1965).

Circadian rhythms have been extensively studied in insects both under field and laboratory conditions as reviewed by HARKER (1961). However, similar observations concerning ticks are rather scanty and reports in the literature are relatively rare.

POMERANTZEV & ALFEEV (1935) and KHEISIN & LAVRENENKO (1956) reported that engorged *Ixodes ricinus* female ticks on cattle detached mainly during day

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time in pasture. SMITH (1945) observed that nymphs of *Ixodes dentatus* tended to drop during the day while larvae mostly at night. SERDYUKOVA (1946), working with *Hyalomma anatolicum*, pointed out that the engorged stages dropped at night, particularly between 21.00–23.00. BALASHOV (1954) studied the daily rhythm of "drop-off" of engorged *Ixodes persulcatus* female ticks. He found that most of the ticks dropped from the cattle early in the morning when the herd was moved out from its night shelter on to the pasture. Similar results were presented by HITCHCOCK (1955) for *Boophilus microplus* engorged females.

While not being able to find such a diurnal rhythm in the "drop-off" of engorged female ticks of *Rhipicephalus appendiculatus*, KITAOKA (1962) described such a phenomenon in the tick *Haemaphysalis bispinosa*. GEORGE (1963) studying the circadian rhythm of the detachment of *Haemaphysalis leporispalustris* engorged female ticks from the rabbit pointed to its being possibly correlated to the diurnal activity of its rabbit host.

As to the "soft ticks" (Argasidae), ENIGK & GRITTNER (1953) found that engorged larvae of *Argas persicus* detached mainly at night.

In the present work the circadian rhythm of the detachment of engorged stages of *Hyalomma excavatum*, *Rhipicephalus sanguineus* and *Argas persicus* have been studied.

Materials and Methods

Laboratory bred larvae, nymphs and adults of *Hyalomma excavatum* (Koch) and *Rhipicephalus sanguineus* (Latreille) have been used throughout this study. The methods of tick infestation, collection and maintenance are described elsewhere (HADANI & CWILICH). Larvae and nymphs were allowed to feed on gerbils whereas adults were reared on rabbits.

In the case of *Argas persicus* (Oken), the larvae only suck blood for some days. Their circadian rhythm was also studied. The ticks were fed on chicken, the beaks and legs of which were tied so as to prevent them from throwing off the larvae (DI-NUR, personal communication).

Infested animals were kept at room temperature and exposed to light : darkness (L.D.), hours ratios as described later.

Engorged ticks, dropping and collected in the pans below the hosts, were counted every 2 hours and the number depicted as percentage of the total number of ticks found throughout the trial.

Results and Discussion

Results are presented in Figures 1, 2 and 3.

It can be seen that well defined patterns in the rate of the detachment of engorged larvae, nymphs and adults of *H. excavatum* and *Rhipicephalus sanguineus* from the gerbil host were obtained. Similar results were recorded for larvae of *A. persicus*.

The "drop-off" of engorged larvae reached a peak at 14.00 and 18.00 for *H. excavatum* and *R. sanguineus* respectively. Similarly highest values of the detachment of engorged nymphs and adults were noted for both species at 2.00 and 22.00 respectively. As to the larvae of *A. persicus* a peak was recorded at 22.00.

The gerbil, being essentially a nocturnal animal (ZEHAVI, personal communication), spends most of the daylight hours in the burrow. Comparing the results obtained in the laboratory to the diurnal behaviour of the gerbil in the field it appears that the animal would tend to drop off most of its larval ticks while

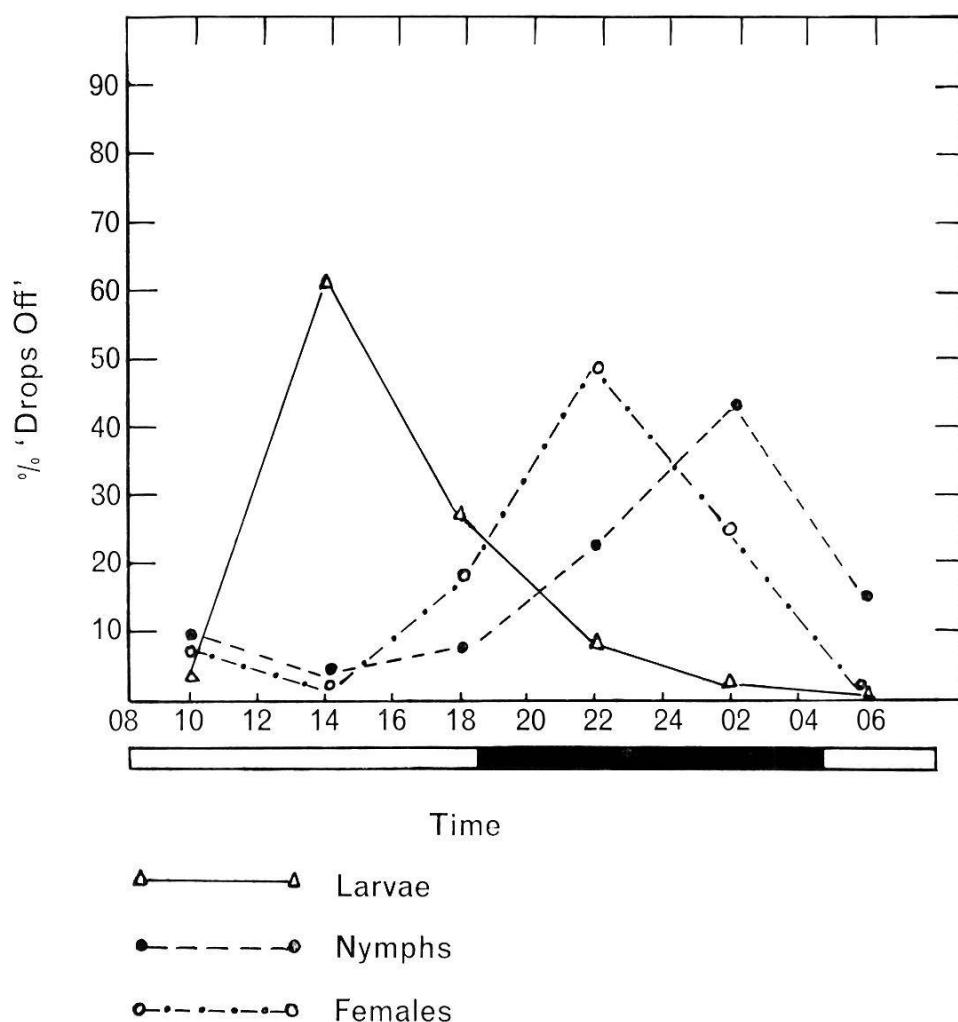


Fig. 1. Pattern of "drop off" of *Hyalomma excavatum* in L.D. 14:10 light cycle.

still in the burrow (peaks of dropping noted at 14.00 and 18.00). Consequently the issuing nymphs will easily find their way to the host sheltered for long hours in its burrow.

The engorged nymphs, on the other hand, tend to detach, once replete, in the early hours of the morning when the gerbils are active in the general habitat outside their burrows. Hence the issuing adults are more favourably located for seeking their way to the bigger mammalian hosts.

The circadian rhythm of detachment of the engorged larvae and nymphs from their gerbil host does seem to overlap that of the diurnal activity of the host — the ticks having adapted themselves so as to increase considerably the chances of host finding and survival.

As to the detachment of the replete females, in both cases, a peak was noted at 22.00. The multiple mammalian hosts harbouring the adult ticks (hedgehogs, porcupines, wild pigs and domestic ruminants) would allow the engorged females, in part at least, to drop in the field and lay eggs in sheltered, vegetation covered areas visited by the rodents which will serve as hosts to the resulting larvae.

As to the larvae of *A. persicus* it looks as if the chicken being active during daytime the detachment of the engorged larvae at night when the birds are inactive might increase their chances of survival.

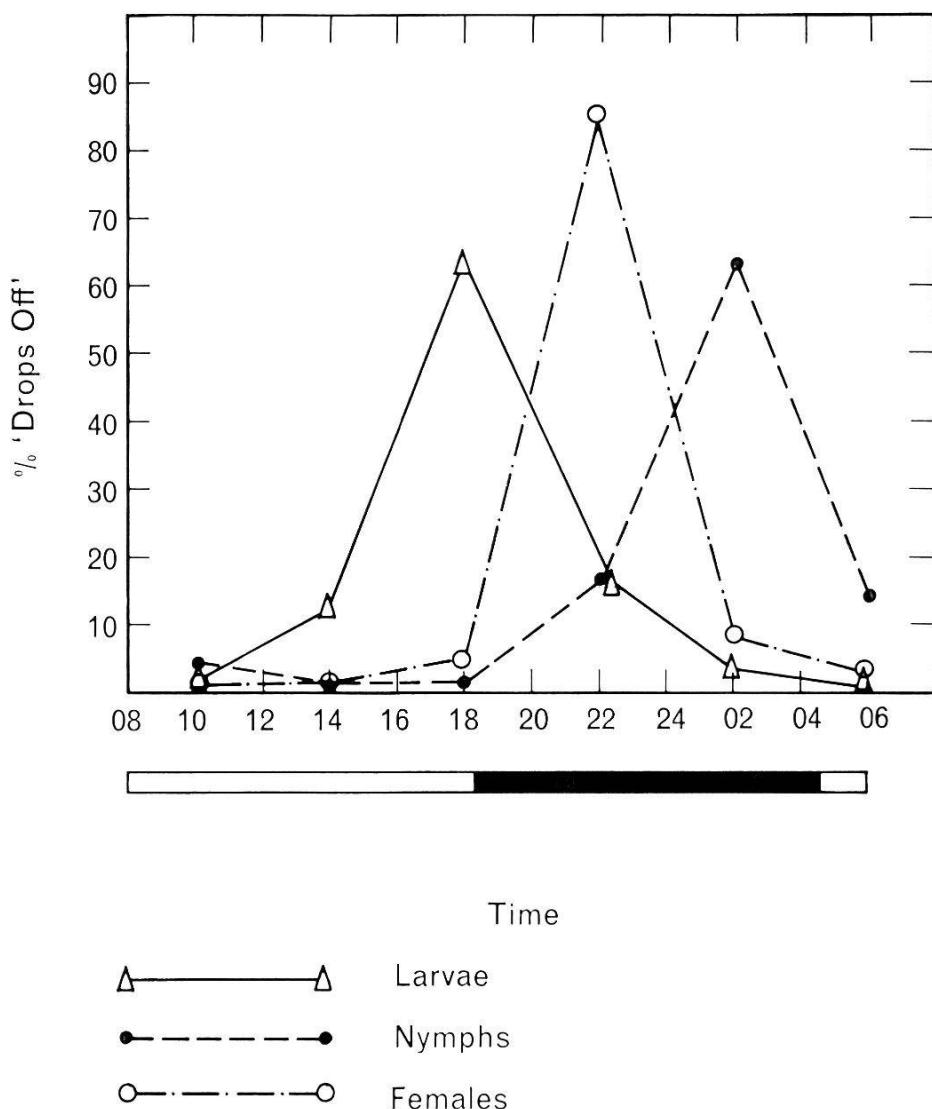


Fig. 2. Pattern of "drop off" of *Rhipicephalus sanguineus* in L.D. 14:10 light cycle.

Previous observations (KITAOKA, 1962) showed that light seemed to stimulate the detachment of engorged females of *Boophilus microplus* and *Haemaphysalis bispinosa* and at the same time to inhibit the final stage of rapid engorgement ("third stage" of KITAOKA) which usually takes place at night. Consequently the effect of different light cycles on the rhythm of tick detachment has been further studied using nymphs of *R. sanguineus*. The results are presented in Figure 4.

It can be seen that varying the ratio of light : darkness hours did not change basically the overall pattern of the detachment engorged nymphs from the gerbils. However, by increasing the hours of darkness the peak of "drop-off" was shifted to the left i.e. 2.00, 22.00, and 18.00 corresponding to L.D. hours ratio of 14:10, 12:12 and complete darkness respectively.

It can be concluded that light does have an effect on the timing of the phase of "drop-off" of engorged nymphs with relation to solar time, though one cannot refer to either stimulatory or inhibitory effects. Furthermore it appears that there is an inherent, endogenous cycle of "drop-off" of engorged stages which might be intimately related to the biological diurnal activity of the gerbil host.

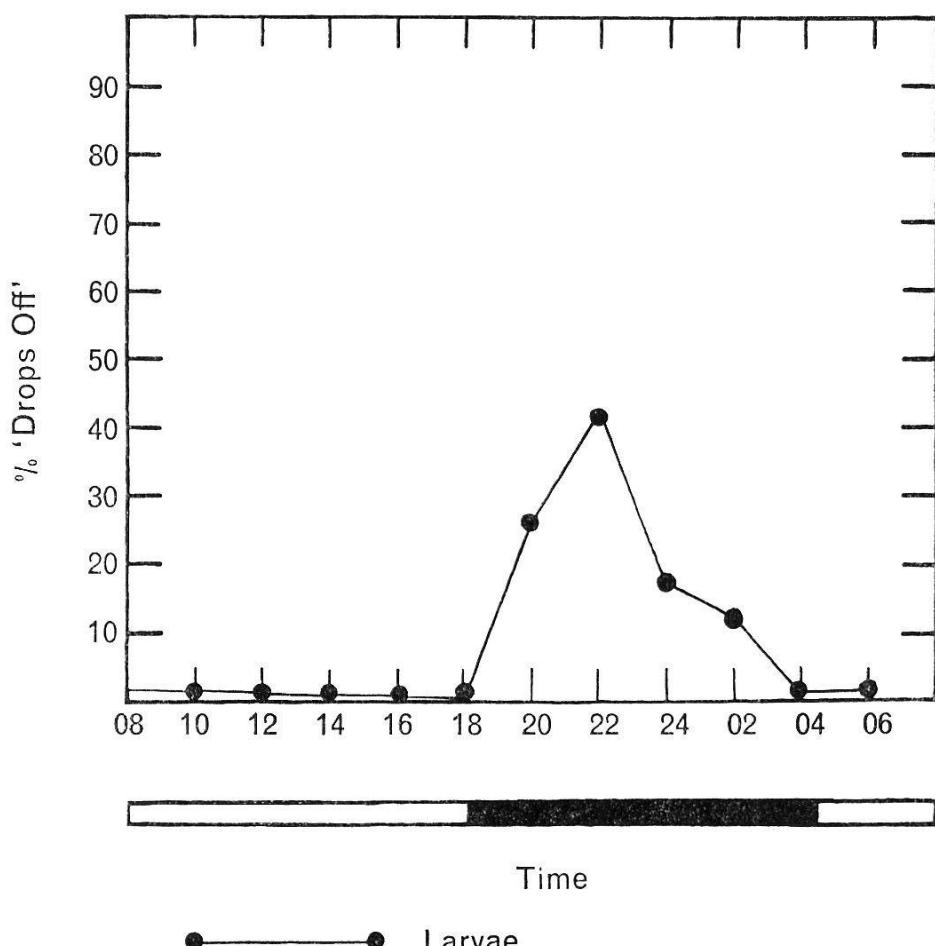


Fig. 3. Pattern of "drop off" of *Argas persicus* in L.D. 14 : 10 light cycle.

The influence of the environment and the behaviour of the host on the circadian rhythm of the "drop-off" of engorged ticks from the host are under study.

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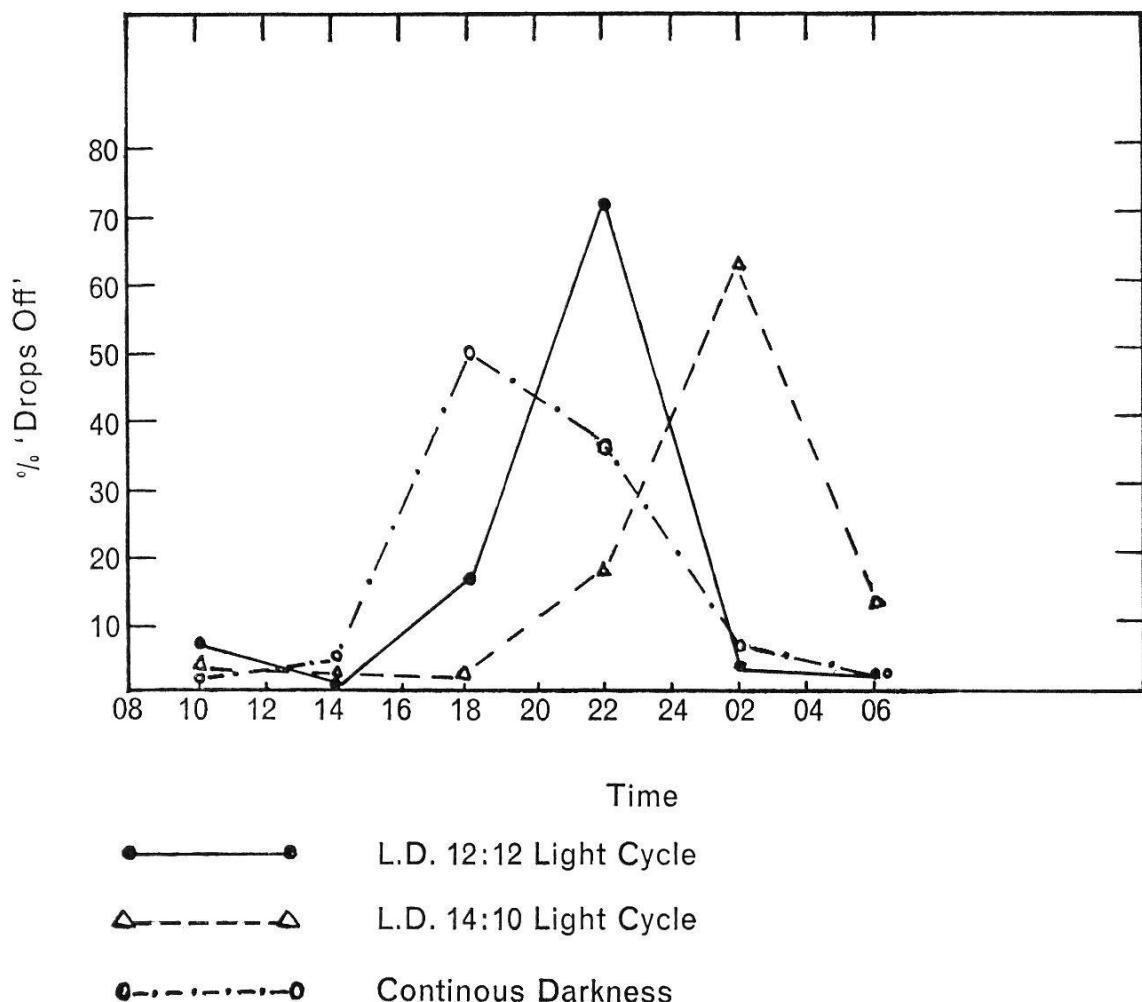


Fig. 4. Pattern of "drop off" of nymphs of *Rhipicephalus sanguineus* in 3 different light cycles.

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Serologische Untersuchungen an mit *Dipetalonema viteae* (Krepkogorskaja 1933) infizierten Hamstern mit Hilfe der Immunofluoreszenzmethode

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Hamster lassen sich mit *Dipetalonema viteae*, einer Nagetierfilarie von Wüstenratten, infizieren. Studien über den Infektionsverlauf sowie Ergebnisse über Makrofilarien-Implantationen und Mikrofilarien-Injektionen beim Hamster sind beschrieben (WEISS, 1969). Hier sollen kurz Ergebnisse serologischer Untersuchungen zusammengefaßt werden. Die indirekte Immunofluoreszenzmethode zur Diagnose von Humanfilariosen nach COUDERT et al. (1968) ist leicht abgeändert übernommen worden. Als Antigen werden 7 μ dicke Kryostatschnitte von *D. viteae* verwendet. Das markierte Anti-Hamster- γ -Globulin stammt vom Institut Pasteur, Paris, und wird auf 1/20 verdünnt. Die schwächste Serumverdünnung, bei welcher eine Fluoreszenz als spezifisch angesehen werden kann, liegt bei 1/20. Die stärkste Serumverdünnung, bei welcher der Test noch deutlich positiv ausfällt, gilt als Antikörpertiter.

Bei einer Gruppe von 12 Hamstern ist der Verlauf des Antikörpertiters bis über ein Jahr nach Infektion untersucht worden. Die durchschnittliche Infektionsdosis hat 110 Larven III pro Tier betragen. Die Abbildung zeigt die Bereiche der reziproken Einzeltiter sowie die Mittelwerte. 4 Wochen nach Infektion lassen sich bei einigen Tieren die ersten Antikörper nachweisen. Es folgt ein Titeranstieg, der 5 bis 6 Monate nach Infektion ein Maximum erreicht. Der Antikörpertiter fällt langsam ab. Ein Jahr nach Infektion liegen die reziproken Titer bei 40 und 80. Zu diesem Zeitpunkt lassen sich neben kalzifizierten noch lebende adulte Filarien nachweisen.

Durch drei intraperitoneale Injektionen von homogenisierten Filarien in reine Tiere innerhalb von drei Wochen wird eine langandauernde Antikörperproduktion stimuliert. 2 Wochen nach der dritten Injektion wird ein Titermaximum erreicht. Bis zu 9 Monaten nach der ersten Injektion lassen sich Antikörper nachweisen.