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Tick-Host Relationships.
II. Factors Affecting the Circadian Rhythm of "Drop off" of Engorged Preimaginal Stages of the Tick *Hyalomma excavatum* (Koch, 1844) from the Gerbil – *Meriones tristrami* *

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In a previous report (HADANI & RECHAV 1969) a circadian rhythm in the "drop off" of engorged stages of *Hyalomma excavatum* and *Rhipicephalus sanguineus* from their gerbil host has been described and discussed in the light of the pertinent literature. The factors affecting these "biological clocks" and the exact nature of their physiological mechanisms are far from being known and understood.

Several authors (see review by HARKER 1961) pointed out the possible effect of various factors such as host movement, light and temperature on these circadian rhythms. BALASHOV (1954), studying *Ixodes persulcatus* has shown the effect of moving the cattle on to the pasture on the rhythm of detachment of engorged females from these animals. The author believed that this phenomenon had to do with a change in light conditions as well.

KITAOKA (1962) found a similar circadian rhythm in the "drop off" of *Haemaphysalis bispinosa* engorged ticks. He suggested that "the primary factor controlling feeding and stimulating dropping of engorged females was the rhythmic change in the intensity of light during the day and the night".

However, GEORGE (1963), studying similar phenomenon in the tick *Haemaphysalis leporis palustris,* considered the circadian rhythm to be essentially endogenous without excluding completely the possible effect of a certain sensory cue on the part of the host. He further showed that changing the feeding time of the host, being maintained in continuous darkness, reversed the rhythm of "drop off".

In the present work some observations on the effect of factors such as light/darkness (L.D.) hours ratio, and host reactions on the circadian rhythm of detachment of engorged preimaginal stages of *Hyalomma excavatum* are reported.

Materials and Methods

Larvae and nymphs of *H. excavatum* feeding on the gerbil – *Meriones tristrami* have been used throughout these trials. The methods of feeding, collection and maintenance of *H. excavatum* larvae and nymphs have been described previously (HADANI et al. 1961, HADANI & RECHAV 1969). Engorged ticks were collected at 2–4 hours intervals. Throughout the experiments described below a total of 140–250 engorged larvae and 30–70 engorged nymphs were collected per gerbil.

Four different L.D. hours ratios have been examined, namely: 14:10, 12:12, continuous light (consisting of daylight and artificial light supplied by G.E. Sunlamps, 275 W, 110–125 V) and continuous darkness.

All the trials were carried out under room conditions at 25 ± 3°C and 60 to 75% R.H.

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Immobilization of the animals has been achieved by the injection of a tranquilizer – "Vetacalm" [1]. The drug was administered at the dose of 0.4 ml per animal and thereafter the animals were kept in this immobilized state by the administration of 0.2 ml "Vetacalm" every 4 hours during the first day and 0.1 ml afterwards till the end of the trial [2].

The ticks, used in the experiments were kept most of the time in a darkened incubator.

Feeding the host animals throughout the trials was carried out at the same time, namely between 7.00–8.00.

Results and Discussion

As mentioned above trials wer carried out at various L.D. hours ratios, while on each occasion both larvae and nymphs were used. The following results were obtained:

1. L.D. light cycle of 14:10 hours

Results given in Figure 1 show a "drop off" pattern reaching a peak at 14.00 and 2.00 hours for *H. excavatum* larvae and nymphs respectively. Similar findings were reported previously (HADANI & RECHAV 1969).

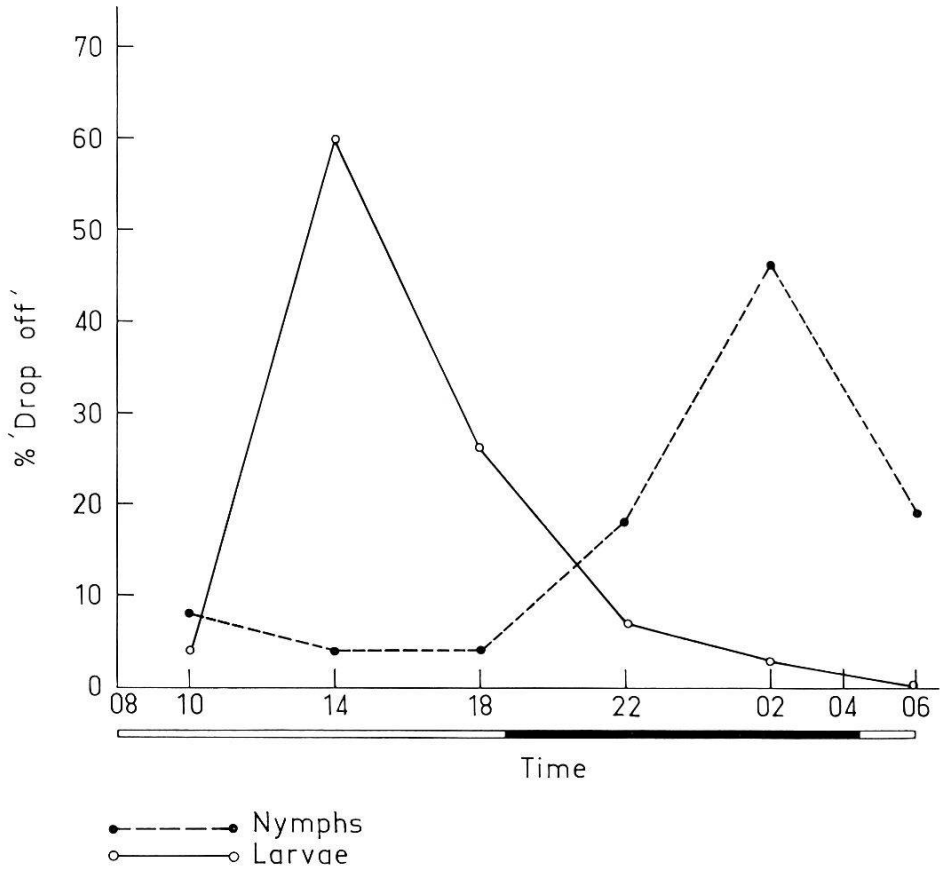

Fig. 1. Pattern of "drop off" of engorged larvae and nymphs of *Hyalomma excavatum* in a L.D. 14:10 light cycle.

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[1] "Veterinaria" A.G. Zürich, Switzerland.

[2] "Vetacalm" has been shown to have no effect on the process of larval and nymphal engorgement per-se.

2. L.D. light cycle of 12:12 hours

When the trials were carried out similarly at a L.D. ratio of 12:12 hours a peak of "drop off" was again recorded between 10.00–14.00 for larvae with a slight tendency to the earlier hours while a definite "shift to the left" was noted for that of the nymphs, reaching a peak between 18.00–2.00 with a maximum at 22.00. Results are summarized in Figure 2.

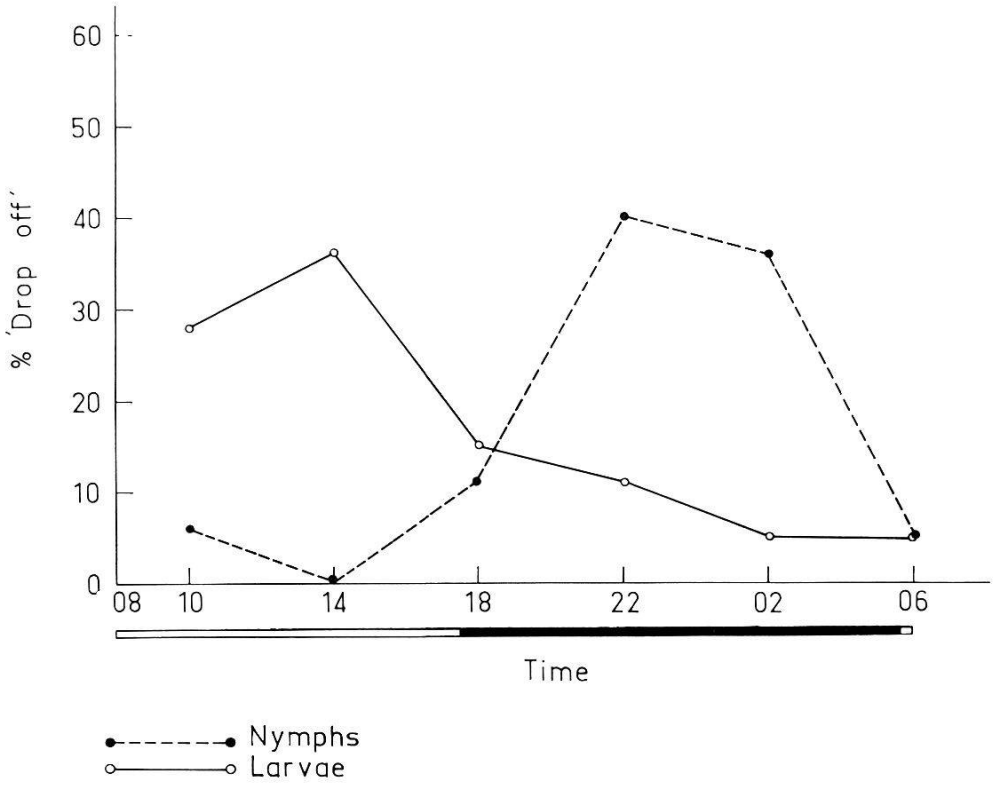

Fig. 2. Pattern of "drop off" of engorged larvae and nymphs of *Hyalomma excavatum* in a L.D. 12:12 light cycle.

The observations described above showed that the peak of "drop off" under our experimental conditions tended to occur earlier, once light is on, when nights grew longer, i.e. 12:12 L.D. hours ratio instead of 14:10. These findings do not seem to indicate a stimulating effect of the light on the detachment of the engorged ticks. On the other hand such a shifting to the left might prove beneficial to the nymphs infesting the gerbil ceasing outdoor activity and getting into its burrow much earlier in the long nights in winter (ZOOK RIMON, personal communication).

Similar phenomenon has been reported by GEORGE (1963) who found that in a L.D. 12:12 hours light cycle larvae of *Haemaphysalis leporis palustris* would tend to drop off the host 5–6 hours after light is on, whereas in a L.D. ratio of 16:8 hours a peak of engorged larvae leaving the host occurred 8–9 hours afterwards.

The following experiments were carried out in an attempt to make this point clearer.

3. Rhythm of "drop off" of H. excavatum engorged larvae and nymphs when infested gerbils were maintained in continuous darkness or light

In this series of trials the "drop off" engorged ticks has been studied simultaneously at 3 different photoperiodic cycles namely, that of 12:12 hours, complete darkness and continuous light.

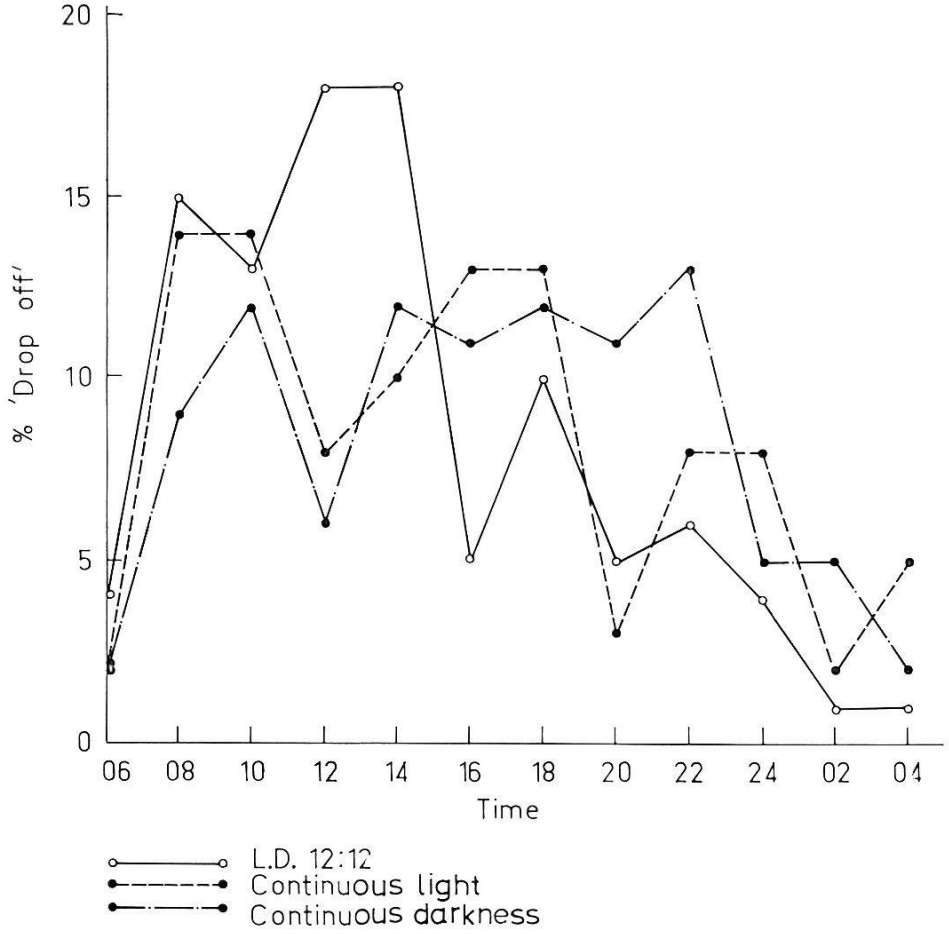

Fig. 3. Pattern of "drop off" of engorged larvae of *Hyalomma excavatum* in 3 different light cycles.

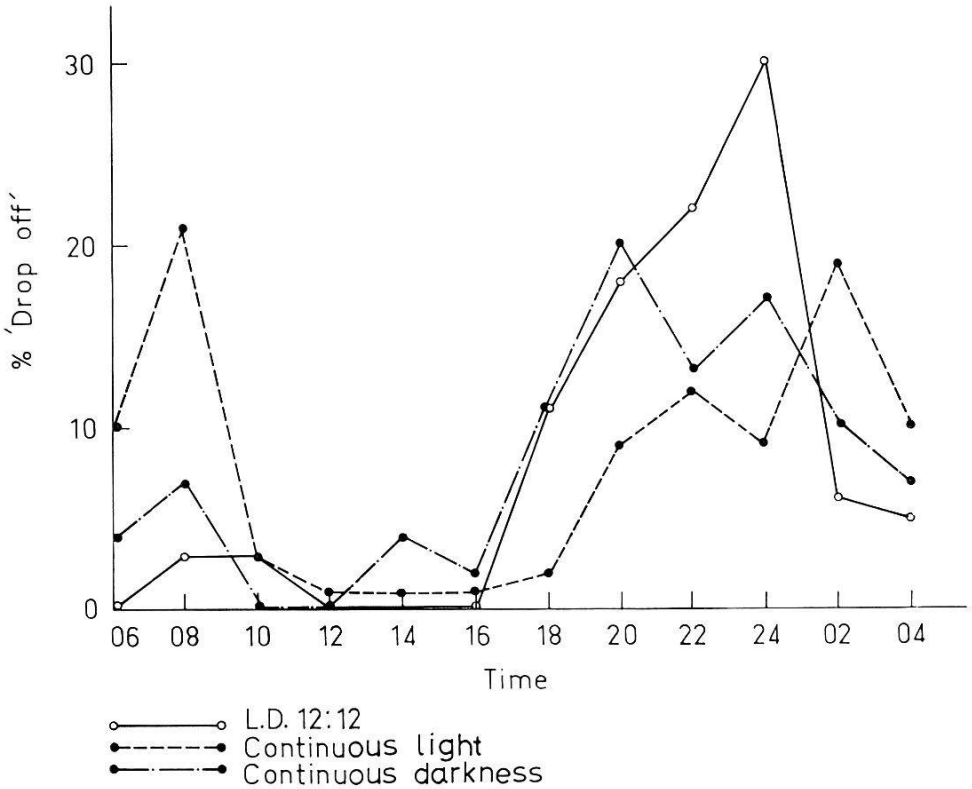

Fig. 4. Pattern of "drop off" of engorged nymphs of *Hyalomma excavatum* in 3 different light cycles.

Results are presented in Figures 3 and 4. Engorged ticks were collected every 2 hours.

Analysis of the results shows that at L.D. 12:12 hours light cycle peaks of detachment of both enorged larvae and nymphs tend to shift to the left. However, continuous light and darkness seem to effect differently *H. excavatum* larvae and nymphs.

As far as the larval stage is concerned the detachment of the engorged ticks would extend considerably throughout the diurnal cycle occurring between 6.00 to 22.00 and decreasing to a minimum in both light and darkness at midnight and the early hours of the morning, i.e. between 22.00–6.00. Slight difference might be noticed in the shape of the "drop off" curves obtained in continuous light and darkness, particularly between 20.00 and 24.00. However, such a difference might be incidental and insignificant.

As to the nymphal "drop off" curve, highest numbers of engorged ticks were always collected around midnight (20.00–2.00) thus conserving the original character of having a peak at night. This finding might further prove the basically endogenous origin sort of "internal clock" of the phenomenon. Interestingly enough, while kept under continuous light, infested gerbils kept shedding engorged nymphs throughout the night hours, i.e. 18.00–10.00. As found in previous trials exposing the infested animals to complete and continuous darkness shifted time range of tick detachment to the left with the engorged nymphs starting to drop in considerable numbers already in the late afternoon (14.00–18.00). Furthermore, notwithstanding the animals being simultaneously infested, engorged nymphs appeared on these animals 20–24 hours before any were collected from the 2 other groups. This observation supports the contention of KITAOKA (1962) that light and darkness might have an effect on the rate of engorgement.

In all, nymphal behaviour seems to be less affected by variations in L.D. hours ratio than the larval one. This fact might have to do either with the nymphs having reached a higher stage of organization or with differences in the respective sensorial systems (DINNIK & ZUMPT 1949).

Futhermore, DE WILDE, in a recent review (1962), emphasizes that "sensitivity to photoperiod is never extended to the whole life cycle . . . in most cases sensitivity is intensified in a limited number of stages or instars".

BALASHOV (1954) emphasized the importance of the effect of the host's movements, such as cattle setting out to pasture in the morning, on the rhythmic "drop off" of *Ixodes persulcatus* engorged females. Some experiments were carried out to check this point.

4. Effect of host reactions on the diurnal pattern of "drop off" of engorged H. excavatum larvae and nymphs

The circadian rhythm of "drop off" of engorged larvae and nymphs of *H. excavatum* has been studied on normally active gerbils and on animals kept practically immobile by the administration of the tranquilizer "Vetacalm" as mentioned above. Both groups of animals have been maintained at L.D. 12:12 light cycle. Engorged ticks were collected and counted every 2 hours.

Results are presented in Figure 5.

As can be seen from the results obtained host's movements had but an insignificant effect on the diurnal rhythm of "drop off" of engorged larvae and nymphs. Consequently the basic endogenous character of the circadian phenomenon described is shown again. As reported by HARKER (1956) and ROBERTS (1966) in the case of *Periplaneta americana,* the endogenous mechanism underlying the circadian phenomenon might depend in our case on periodical rhythmic secretion of a hormone by neurosecretory cells. However, as mentioned before

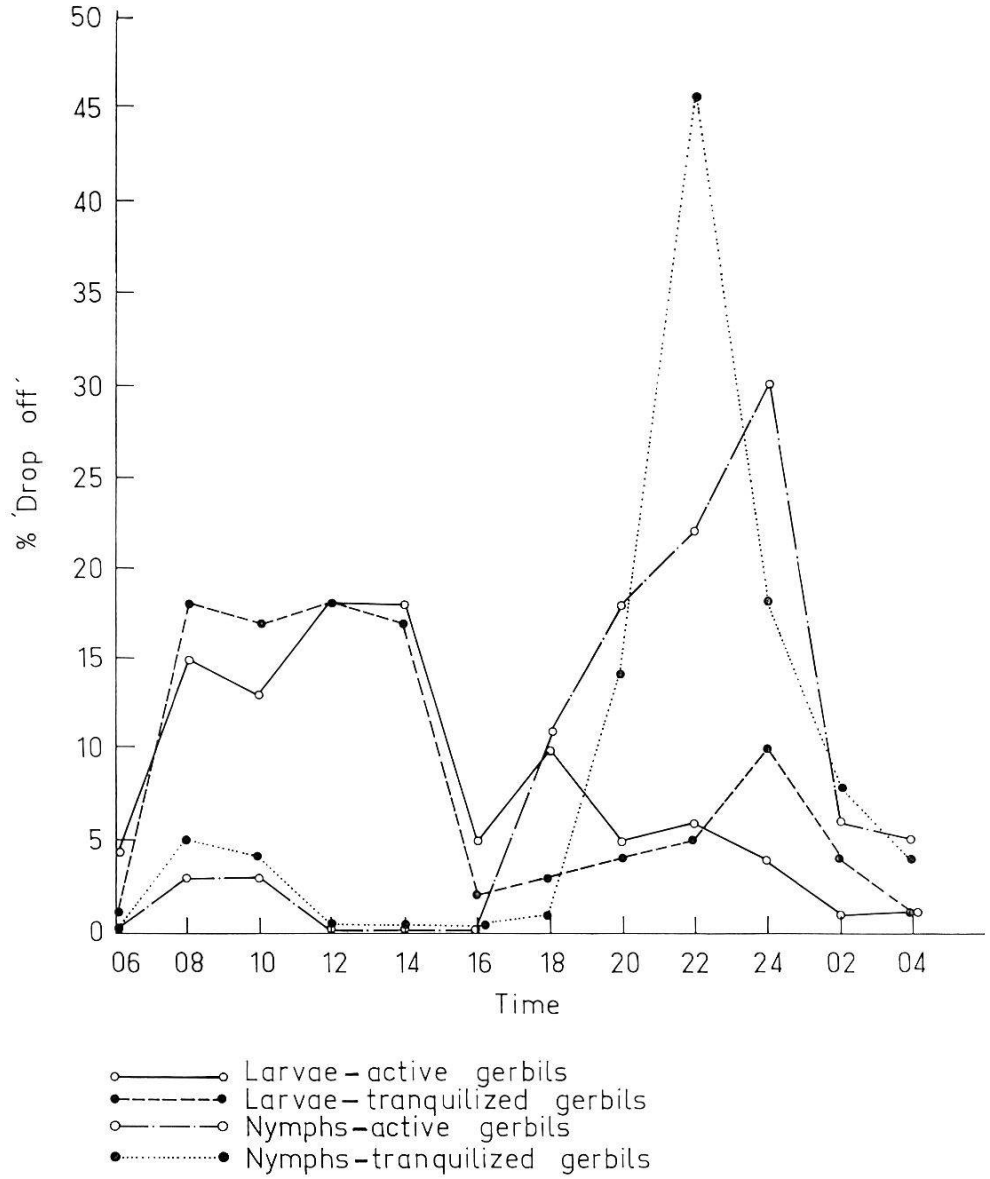

Fig. 5. Pattern of "drop off" of engorged larvae and nymphs of *Hyalomma excavatum* from active and tranquilized gerbils in a L.D. 12:12 light cycle.

one should not exclude the contributing effect of exogenous factors such as temperature, humidity, host's nutrition and reactions and particularly light.

The host *Meriones tristrami* being essentially a nocturnal animal, has been shown to start back to its burrow earlier when nights grew longer and colder i.e. in wintertime. Under such a situation any "shift to the left" in the circadian rhythm of "drop off" of engorged nymphs might be beneficial for the survival of the ticks.

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