Effect of carbon dioxide on the molting activity of two tick species in the laboratory

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Abstract
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Keywords
Amblyomma maculatum, Amblyomma americanum, carbon dioxide, ecdysis

Disciplines
Animal Sciences | Biology | Entomology | Laboratory and Basic Science Research | Physiology | Structural Biology

Comments
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EFFECT OF CARBON DIOXIDE ON THE MOLTING ACTIVITY OF TWO TICK SPECIES IN THE LABORATORY

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ABSTRACT

The effects of carbon dioxide concentration on the molting activity of Amblyomma americanum (L.), and Amblyomma maculatum Koch were investigated. In both species, replete nymphs held in humidity chambers at a high carbon dioxide concentration (ca. 900 ppm) had a 2-4 day delay in peak ecysis when compared to nymphs held at a low carbon dioxide concentration (ca. 400 ppm). In contrast, replete larvae showed no apparent delay in peak ecysis as a result of increased carbon dioxide concentration. Percent total eclosion was not affected by carbon dioxide concentration in either nymphs or larvae.

INTRODUCTION

Previous studies have indicated the importance of maintaining certain environmental parameters to insure successful tick molting in the laboratory. Sacktor et al. (1948) calculated an optimum temperature of 30°C and a relative humidity of 85-100% for molting in Amblyomma americanum (L.). This work was supported by Lancaster and McMillian (1953) who found that engorged A. americanum larvae failed to molt at humidities below 70%. Although optimum temperature and humidity parameters have been determined for laboratory tick rearing systems, the effects of gaseous components on tick molting have not been investigated. It is known that carbon dioxide (CO₂) has an attractant or stimulatory effect on many insects and mites (Garroía 1962, DePoliart and Morris 1967, Miles 1968, Hair et al. 1972) but when concentrations exceed a certain threshold many arthropods are inactivated (Williams 1946, 1957). The objective of this study was to determine the effect of carbon dioxide (CO₂) concentration on the molting activity of two tick species in the laboratory.

MATERIALS AND METHODS

Experiments were conducted on larval and nympha1 Gulf Coast ticks, A. maculatum Koch, and lone star ticks, A. americanum (L.), which had fed to repletion on domestic rabbits. A total of 2000 ticks of each stage and species were collected immediately after drop-off and placed in 0.236L cardboard containers (200 ticks/container) covered with Saran Wrap. Containers were equally distributed in sealed 61 x 41 x 32cm high plexiglass humidity chambers (5 containers/chamber) designed to regulate either a low or high, chamber, CO₂ concentration.

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A low CO₂ concentration (ca. 400ppm) was maintained by circulating atmospheric air through the chamber using a 1/82 hp Dyno-pump (Fisher Scientific Comp.). This pump, positioned adjacent to the chamber, was partitioned between 2 sections of 6mm O.D. plastic tubing. One section was inserted through the side of the chamber, while the second section led to the outside of the laboratory, with the intake positioned 1.2m above ground level. A high CO₂ concentration (ca. 900ppm) was maintained by not circulating atmospheric air through the chamber. Also, the diffusion of laboratory room CO₂ concentration, plus CO₂ liberated from tick respiration helped produce a high CO₂ concentration within the chamber.

The temperature and humidity within each chamber was checked daily prior to CO₂ measurements or tick observations with YSI Tele-Thermometer and YSI Dew Point Hygrometer (Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio). Carbon dioxide was measured between 1000-1100 h with a Model 755-010 infrared spectrophotometer (Infrared Industries, Inc.). Both chambers were held at room temperature (26±1°C), with a relative humidity of 90-4% (Winston and Bates (1960), and a 10L:14D photoperiod. The concentration of CO₂ in the room varied during a 24 h period but remained within a 600-800 ppm range.

After the initiation of tick eclosion, newly molted ticks were counted daily, placed in separate containers and returned to the test chamber. The effect of chamber opening on temperature, humidity and CO₂ concentration was determined by monitoring these parameters at 5 min intervals with the instruments previously mentioned. Counts continued until all molting activity had ceased. Statistical analysis was performed using analysis of variance and Chi-square tests.

RESULTS

The daily dynamics of CO₂ concentration in each chamber, are shown in Fig. 1. The CO₂ concentration fluctuated in both chambers throughout the study but more stability in CO₂ concentration was achieved in the chamber receiving atmospheric air.

Amblyomma americanum nymphs held at low CO₂ concentration began eclosion to adults 14 days after repletion (Fig. 2). Peak eclosion occurred on day 16 and steadily declined until day 22. Eclosion to adults of nymphs held at a high CO₂ concentration began 15 days after repletion, reached a peak on day 20 and rapidly declined until day 25. Significant differences (P<0.05) were observed on days 16, 17, 20 and 21. A total eclosion of 97% was achieved at both low and high CO₂ concentration, with 50% occurring within ±1 day of peak eclosion (Table 1).

A similar pattern was observed in nymphal A. maculatum (Fig. 2). Ticks held at a low CO₂ concentration began eclosion to adults 13 days after repletion and reached a peak on day 18. Eclosion was not observed after day 23. Nymphs held at a high CO₂ concentration began eclosion on day 13 but did not reach a peak until day 20. Eclosion was not observed after day 27. Significant differences (P<0.05) were observed on days 21 and 22. A total eclosion of 96% was achieved at both low and high CO₂ concentration with 60% occurring within ±1 day of peak eclosion (Table 1).

Larval A. maculatum began eclosion to the nymphal stage 8 days after repletion at the low CO₂ concentration and 9 days after repletion at the high CO₂ concentration (Fig. 3). Both reached a peak on day 10 and declined until day 13. Significant differences (P<0.05) were observed on days 9 and 11. A total eclosion of 94% was achieved at both low and high CO₂ concentrations, with 80% occurring within ±1 day of peak eclosion (Table 17).

Chi square analysis showed significantly different (P<0.001) molting patterns between larvae and nymphs held at low compared to high CO₂ concentrations in both tick species.

The effect of opening chambers on temperature, humidity and CO₂ was negligible, since entry into the chambers required only a slight inclination of the glass top. After a 5-10 second opening of a chamber top, the humidity never dropped more than 1% and temperature values never increased more than

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FIG. 1. Daily dynamics of CO₂ concentration in rearing chambers during molting of Amblyomma americanum and A. maculatum.
FIG. 2. Effect of CO$_2$ concentration on eclosion of replete nymphs. SD ± 0.5 are not shown (●).
FIG. 3. Effect of CO$_2$ concentration on eclosion of replete larvae. SD ± 0.5% are not shown (●).
TABLE 1. Percent Eclosion of Ticks Held at Low and High Carbon Dioxide Concentrations.

<table>
<thead>
<tr>
<th>Replete stage</th>
<th>CO₂ Concentrationa/</th>
<th>Eclosion +1 day of peak (1%)</th>
<th>Totalb/ eclosion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nymph</td>
<td>low</td>
<td>50.7</td>
<td>97.3</td>
</tr>
<tr>
<td>nymph</td>
<td>high</td>
<td>57.6</td>
<td>97.7</td>
</tr>
<tr>
<td>larva</td>
<td>low</td>
<td>81.7</td>
<td>94.5</td>
</tr>
<tr>
<td>larva</td>
<td>high</td>
<td>89.8</td>
<td>96.0</td>
</tr>
</tbody>
</table>

A. americanum

A. maculatum

nymph  low  66.6  96.3
nymph  high  61.1  96.5
larva  low  89.2  94.4
larva  high  80.5  93.5

a/  Low = ca. 400 ppm CO₂; High = ca. 900 ppm CO₂.

b/  Based on 1000 ticks.

1°C. The ambient room temperature remained ca. 2°C higher than closed tick chamber temperature. Carbon dioxide concentration in chambers was not recorded after each opening but periodic measurements revealed a decrease of 25 ppm and an increase of 100 ppm in the high and low CO₂ chambers, respectively. After a chamber was opened, the length of time required for temperature, humidity and CO₂ concentration to return to test conditions was ca. 1 h, 30 min and 15 min, respectively.

DISCUSSION

Molting in insects is controlled by an integrated neuro-endocrine system (Wigglesworth 1974). After repletion, nerve impulses stimulate neurosecretory cells of the brain to produce a hormone which passes along axons to the corpora cardia cum and is then liberated into the blood. This brain hormone stimulates the prothoracic glands to produce the molting hormone, ecdysone, which initiates the complex process of ecdysis. Although little is known concerning this process in ticks, recent findings of the presence of ecdysone in Amblyomma hebraeum Koch nymphs indicate hormonal control of molting may be homologous with that of insects (Delbecque et al. 1978). Results of our study indicate that in the presence of high concentration of CO₂, this process is delayed when nymphal ticks eclosed to adults (Fig. 2). Since CO₂ acts on the nervous system to reduce the amount of neurotransmitter liberated and impair the sensitivity of the target organ (Hoyle 1960), this delay may possibly be the direct result of nervous inhibition of hormone secretion due to an increase in CO₂ concentration. Therefore, replete nymphs subjected to CO₂ concentration may have had a slightly reduced release of hormone, thus causing a delay in ecdysis. This conclusion was also postulated by Knight and Gunn (1945) who found growth and molting delayed up to 40% when several *P. tectus* Boie were reared in the same container.
Prolonged exposure to a high concentration of \( \text{CO}_2 \) also increases the ventilatory rate and open spiracular period (Miller 1960, Chapman 1971). Since the prothoracic glands are closely associated with the tracheal system (Wigglesworth 1974), \( \text{CO}_2 \) may be able to exert its effects directly and continuously on the neuro-endocrine system during the molting period.

Although Chi-square analysis showed significantly different (P<0.001) molting patterns between larvae held at low compared to high \( \text{CO}_2 \) concentration, a distinct delay in ec dysis was not apparent (Fig. 3). However, since larval ticks lack a tracheal system and must rely on cutaneous respiration (Balashov 1972), \( \text{CO}_2 \) may not successfully inhibit the neuro-endocrine system and delay ec dysis. Also, the amount of hormone required to initiate ec dysis in larvae may not be as great as in nymphs. Therefore, when replete larvae were subjected to high \( \text{CO}_2 \) concentration, they may have had a slightly reduced release of hormone, the concentration was still sufficient enough to allow ec dysis to proceed without apparent delay.

In summary, our results indicated the importance of \( \text{CO}_2 \) concentration on tick molting. These findings may result in utilization of rearing systems which incorporate a low \( \text{CO}_2 \) environment in conjunction with other essential environmental parameters reducing tick molting time. However, further tests should be conducted to determine if other physiological or metabolic processes are affected by prolonged exposure to high levels of \( \text{CO}_2 \).

LITERATURE CITED


