

CHANGES IN CRITICAL TEMPERATURE DURING
NYMPHAL AND ADULT DEVELOPMENT IN THE
RABBIT TICK, *HAEMAPHYSALIS LEPORISPALUSTRIS*
(ACARI: IXODIDES: IXODIDAE)

By MINH-TAM BUI DAVIS*

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INTRODUCTION

As an adaptation to terrestrial life, the cuticle of terrestrial arthropods has very low permeability to water (Barton-Brown, 1964; Ebeling, 1964), but permeability is enormously increased if the environmental temperature is raised above a certain critical value. This critical temperature, usually different from the lethal temperature, has been recorded for many arthropods (Lees, 1947; Wigglesworth, 1945; Davies & Edney, 1952; Beament, 1959; Oloffs & Scudder, 1966). Although a critical temperature of 52 °C has been shown in *Hyalomma dromedarii* (Hafez, El-Ziady & Hefnawy, 1970), critical temperatures of other ixodid ticks which have been studied are in the range of 32-45 °C. These, in general, are lower than those of the argasid ticks (63-75 °C) (Lees, 1947). Critical temperature thus varies with different species of arthropods, and preliminary studies have shown that critical temperature also varies with age and developmental stage within one species (Wigglesworth, 1945; Nelson & Camin, 1967).

In this investigation changes of critical temperature during the developmental period from nymphal to adult engorgement were measured in the rabbit tick, *Haemaphysalis leporispalustris*, and the relationships of developmental stage, engorgement, age and sex to these changes were analysed.

MATERIALS AND METHODS

Animals

Nymphs and adults of the rabbit tick, *Haemaphysalis leporispalustris* (Packard), were used in this investigation. Engorged nymphs were tested at 1, 4 and 15 days after drop-off (i.e. after completing engorgement and dropping from host). Unengorged adults were tested at 7 days and at 1-2 months after ecdysis. Fully engorged adult females were tested one day after drop-off. Partially engorged adult females were tested just prior to the rapid engorgement phase, when they had been on the rabbit for 10-14 days and their body weights had gradually risen from about 1.2 to 40-77 mg. Within the next 1 or 2 days (rapid engorgement phase) the body weights usually rise abruptly to about 223 mg and engorgement is completed.

Ticks were reared on domesticated rabbits. The engorged ticks were collected from

* Department of Physiology and Cell Biology, University of Kansas, Lawrence, Kansas 66044.
Present address: Department of Biochemistry, Washington University School of Medicine, St Louis, Missouri 63110.

Table 1. *Mean weight (mg) of individual ticks of different ages*

Developmental stages	Mean \pm s.d.	Range	n
Engorged nymphs 1 day after drop-off	2.743 \pm 0.095	2.333-2.893	336
Engorged nymphs 4 days after drop-off	2.564 \pm 0.083	2.410-2.769	186
Engorged nymphs 15 days after drop-off	2.152 \pm 0.077	1.943-2.293	314
Unfed females 7 days after ecdysis	1.329 \pm 0.072	1.128-1.494	1076
Unfed females 1-2 months after ecdysis	1.200 \pm 0.061	1.110-1.391	1097
Unfed males 1-2 months after ecdysis	0.761 \pm 0.043	0.655-0.888	552
Partly engorged females	58.971 \pm 9.850	40.640-76.976	37
Fully engorged females 1 day after drop-off	223.438 \pm 55.030	159.568-359.673	43

n = number of ticks.

a water-filled pan beneath the rabbit cage within 24 h after drop-off in early experiments and within 9 h in later experiments. The ticks were then washed with cold water and placed on absorbent paper to be partially dried. All engorged ticks, except some of the nymphs, were kept at room humidity for 1 day before being stored in clear plastic vials (2.5 \times 3.2 cm = h \times d) at 25 °C and 85% relative humidity (R.H.). Nymphs, which were to be tested 1 day after drop-off, were stored at 85% R.H. immediately after their collection. The humidity was controlled by a saturated solution of potassium chloride (KCl) (Winston & Bates, 1960). Partially engorged females were pulled from the host with their mouthparts undamaged and stored at 85% R.H. for 1 day prior to the experiment.

Only ticks of a certain size were selected for this investigation (mean weights in Table 1). Coefficients of variation in weight were within 5.42% for engorged nymphs and unfed adults and within 16.70-24.63% for engorged adult females.

As HCN was found to lower critical temperature (CT) (Davis, 1973*b*), it was decided that live ticks should be used in the determination of CT. Arguments against the use of live ticks for CT determination are that activity and metabolic rates will vary with temperature and possibly affect CT, and that active inhibition of water loss might mask or distort measurements of CT. Some of these objections, however, could be minimized. (1) Ticks were kept immobile during experiment by wrapping in perforated aluminum foil. (2) The CTs of *H. leporispalustris*, except for engorged nymphs 15 days after drop-off, are fortunately far below the lethal temperature (50 °C for engorged nymphs and adults exposed to 0% R.H. for 30 min). Therefore measurements of CT are not complicated by the sudden increase of water loss at death. (3) Increase in respiration and ventilation, leading to an increase of water loss, has been observed in the desert scorpion (Hadley, 1970), the whip scorpion (Ahearn, 1970) and the locust (Loveridge, 1968). The increase of respiration and ventilation with high temperature, together with active inhibition of water loss at low temperature, might enhance the magnitude of CT break but did not appear to affect CT *per se*. After considering the advantages and disadvantages of using live and dead ticks in the determination of CT, it was concluded that the use of live ticks was preferable to the usual practice of employing dead ticks because, whereas the effects of killing agents are either unknown or unavoidable, the disadvantages associated with living ticks can be minimized.

Determination of critical temperature

Critical temperature was determined as the temperature at which the curve of the rate of water loss against temperature (25–50 °C) showed a sharply defined discontinuity.

At each constant temperature different groups of three or six ticks were exposed for 30 min to 0% relative humidity. The rate of water loss was calculated from the difference in the ticks' weight before and after the exposure and expressed as $10^{-1} \times \mu\text{g}/\text{mmHg}$ saturation deficit/30 min/tick. Data were discarded if the engorged females defecated.

The humidity (0%) was controlled by drierite (CaSO_4). The ticks were weighed with a Cahn electro-balance, model G-2, in the selected range of 0–10 mg.

The apparatus which provided constant temperature and humidity is described as follows. A metal can (8.5 × 7 cm = d × h) containing drierite was used as a humidity chamber (Fig. 1). Through the plastic lid of the chamber were inserted three tick containers and one glass thermometer. The tick container was made from a screen vial with plastic frame (12.5 × 23 mm = d × h) and a long glass tube (10 × 0.6 cm = l × d). Ticks were introduced into the container through this tube (Fig. 1). The tip of the thermometer and the ticks, which were restricted to the bottom of the container, were at the same height, 3.85 cm below the lid of the chamber.

A Pyrex cylinder with a Styrofoam cover was used as a water bath. The humidity chamber was weighted down by a bag of lead pellets and immersed in the bath so that the water level was 1.5 cm from the top of the chamber. The temperature of the water was maintained uniformly by a temperature controller (model 71, YSI) and a magnetic stirrer. In order that variation in the water loss results might be avoided, the desired temperature in the humidity chamber was reached gradually rather than overshoot and then corrected.

In early experiments ticks were not restricted to the bottom of the tick chamber and settled on the underside of the chamber lid. The ticks were thus exposed to temperatures that were found to be lower than those recorded by the thermometer. In later experiments individual ticks were confined in perforated aluminium pans within the tick chamber. The confinement had negligible effects on the rate of water loss because the same rate of water loss at 47 °C was obtained in engorged nymphs, 15 days after drop-off, whether the ticks were confined in aluminium pans or not ($P > 0.05$, Wilcoxon two-sample test). The nymphs were immobile 15 days after drop-off and thus suitable for this test.

Statistical methods

The various statistical tests used are described by Sokal & Rohlf (1969).

RESULTS

During the developmental period from nymphal to adult engorgement, critical temperature (CT) changed with developmental stage and nutritional state but did not differ between sexes.

One day after drop-off engorged nymphs lacked a well-defined CT; only a gradual

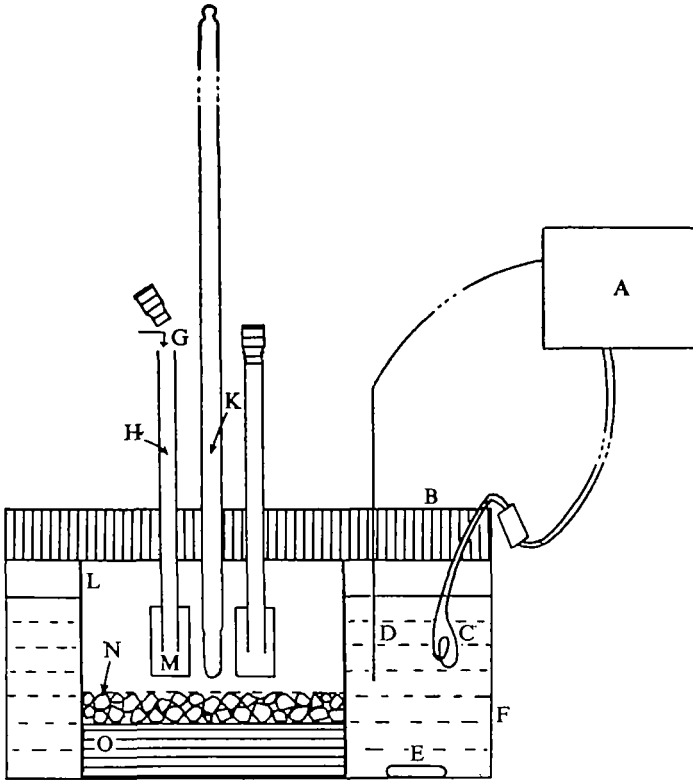


Fig. 1. Diagram of apparatus used to control humidity and temperature. A, Temperature controller; B, Styrofoam cover; C, heater; D, probe thermometer; E, magnetic stirrer; F, water bath; G, entrance to tick container; H, glass tube; K, glass thermometer; L, humidity chamber; M, tick container; N, drierite; O, lead pellets.

break over the range from 36 to 40 °C was detected (Fig. 2), but it had become more distinct 4 days later, at 40–41 °C. Nymphal CT continued to increase during the following 11 days and had reached 46–47 °C by 15 days after drop-off. Critical temperature changed again when the nymphs moulted into adults (Fig. 3), to 42–43 °C in females 7 days after ecdysis, and remained constant for at least 2 months after ecdysis. Males had the same CT as females during this period. Just prior to the rapid engorgement phase, after the females had become engorged to the weight of 40–77 mg, CT had decreased from 42–43 to 37–38 °C. Preliminary data indicated that this change did not occur until just before the rapid engorgement phase. Critical temperature had further decreased to 35–36 °C 1 day after drop-off (160–360 mg) (Fig. 4).

As CT increased with age during nymphal development, the rate of water loss decreased with age at 35–36 °C ($P < 0.005$, Kruskal–Wallis test and Fig. 2). However, at 25 °C the rate of water loss was unaffected by age, whether the loss was expressed as rate per tick or rate per mg of body weight ($P > 0.05$, Kruskal–Wallis test and Fig. 2). The change in the rate of water loss may have been due to the amount of active resistance to water loss through the cuticle and/or the spiracles, or to a change in the chemical composition of the cuticle. At 50 °C, a temperature above the critical temperature, the nymphs were five times more permeable at 1 day than at 15 days after drop-off (59.466 ± 5.134 v. 11.098 ± 1.738 (10^{-1}) $\times \mu\text{g}/\text{mmHg}/30$ min/tick).

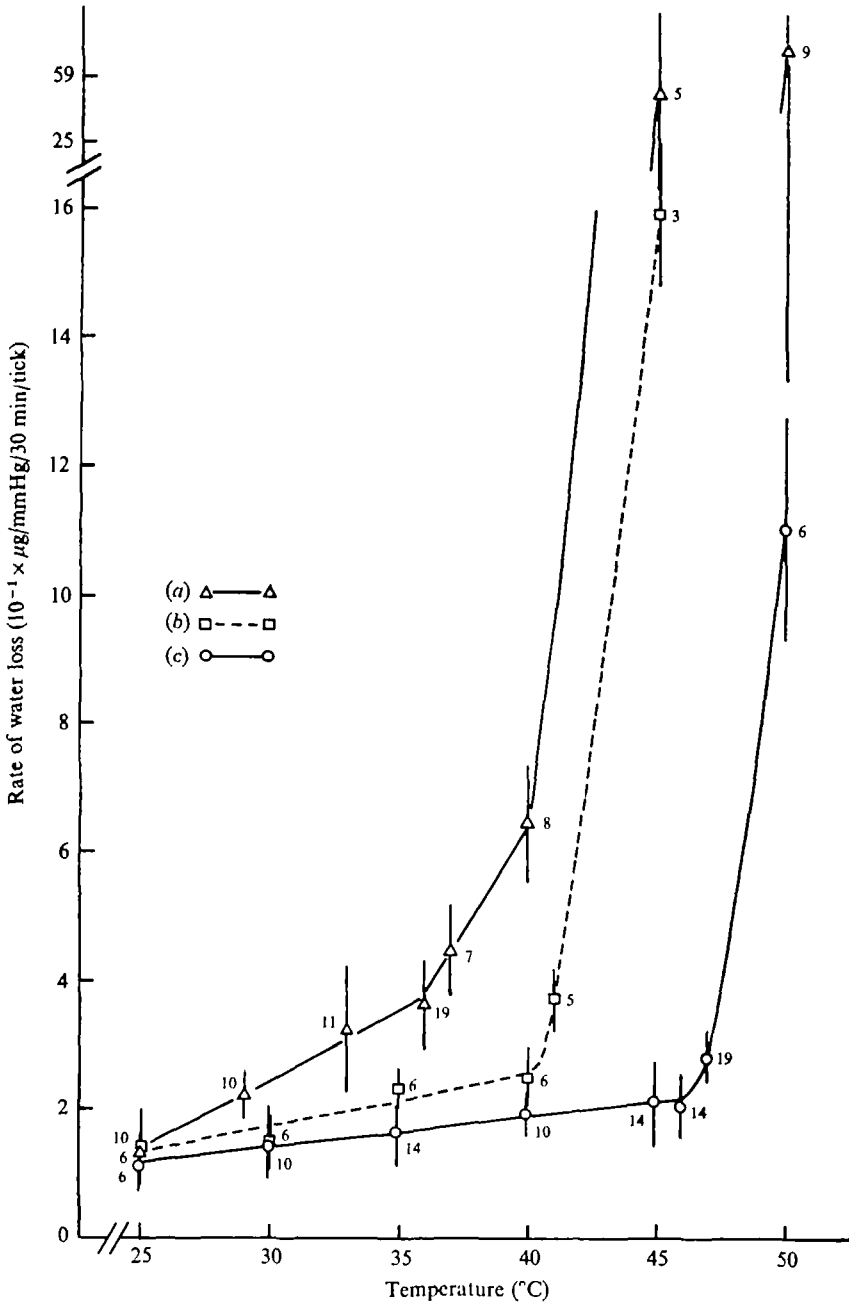


Fig. 2. Rate of water loss/temperature for engorged nymphs. *a*, Engorged nymphs 1 day after drop-off; *b*, engorged nymphs 4 days after drop-off; *c*, engorged nymphs 15 days after drop-off. Vertical lines represent the standard deviations of the means. The numbers next to the means represent the numbers of replicates, with 3 or 6 ticks per replicate.

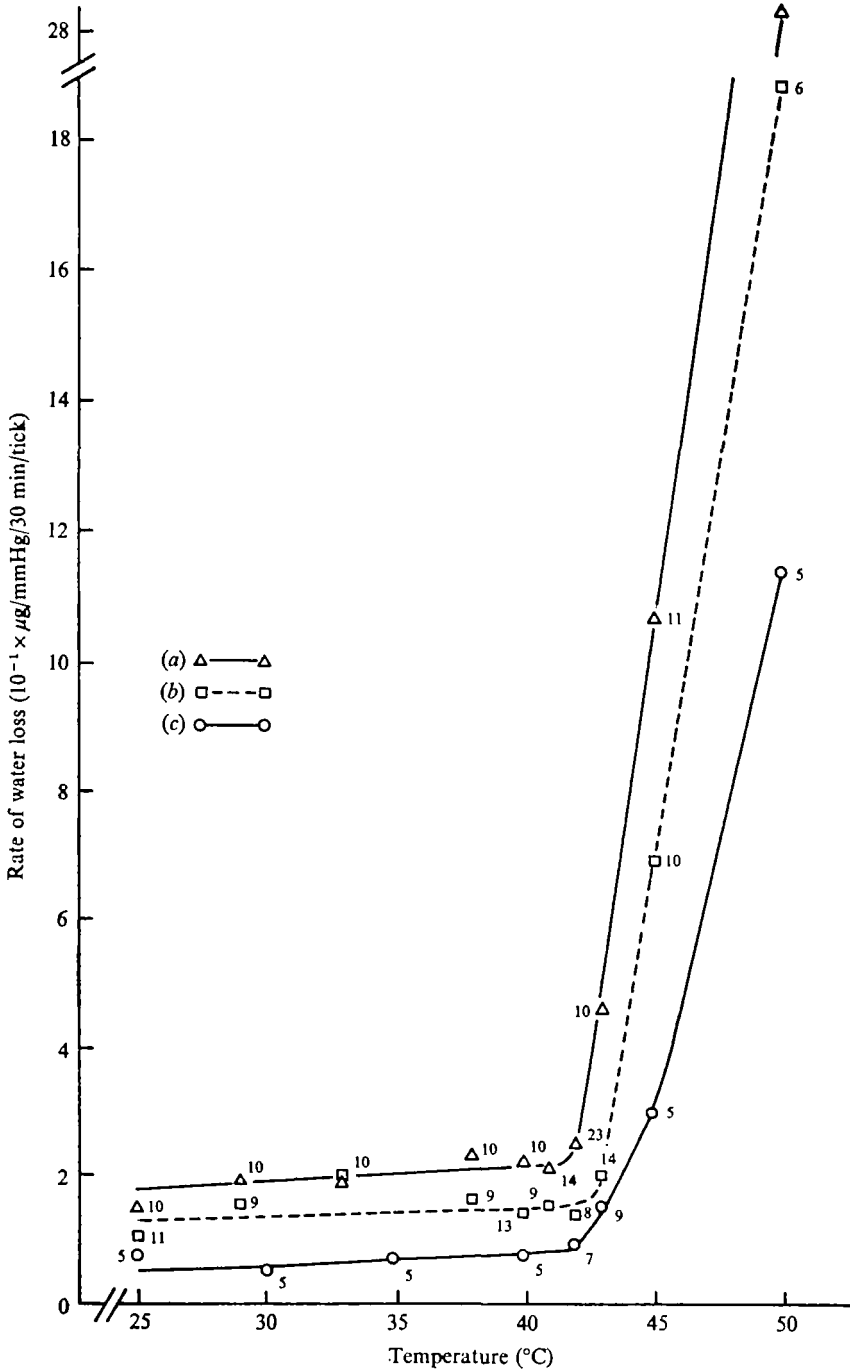


Fig. 3. Rate of water loss/temperature for female and male ticks. *a*, Females 7 days after ecdysis; *b*, females 1-2 months after ecdysis; *c*, males 1-2 months after ecdysis. The numbers next to the means represent the numbers of replicates, with 6 or 12 ticks per replicate.

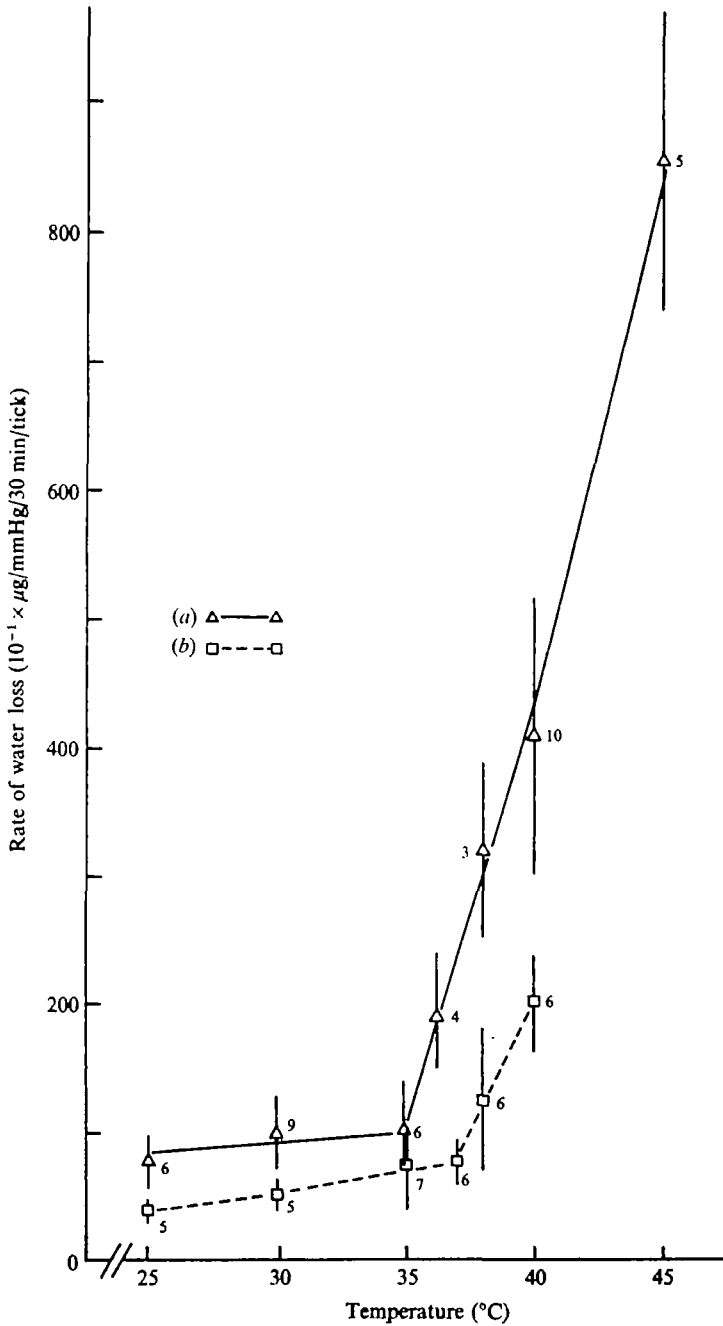


Fig. 4. Rate of water/temperature for engorged female ticks. *a*, Fully engorged females; *b*, partly engorged females. Vertical lines represent standard deviations of the means. The numbers next to the means represent the numbers of replicates, with 1 tick per replicate.

The curve of water loss/temperature for adult males was slightly less steep than that for females. When the water loss was expressed as rate per mg of body weight rather than per tick, similar rates of water loss were obtained for females and males at 25 °C ($P > 0.05$, Wilcoxon two-sample test and Fig. 3). At 40 and 50 °C there were differences in the rate of water loss associated with age, but not with sex ($P < 0.01$ at 40 °C, $P < 0.001$ at 50 °C, Wilcoxon two-sample test and Fig. 3).

Both CT and rate of water loss in adult females decreased with engorgement at 25 °C ($P < 0.05$, Kruskal-Wallis test). These results were obtained when allowance for the difference in weight of the ticks had been made, and the rate of water loss was thus in $(10^{-1}) \times \mu\text{g}/\text{mmHg}/30 \text{ min}/\text{mg}$ body weight. The decrease in permeability with engorgement has also been reported in some other ticks (Belozerov, 1967).

At 37 °C (0% R.H.), which is probably somewhat above skin temperature of the host, the rate of water loss of engorged adults was about 600 $\mu\text{g}/30 \text{ min}/\text{tick}$. Thus, for 24 h of rapid engorgement, the highest amount of transpiration would be about $600 \mu\text{g} \times 48 = 28.8 \text{ mg}$, or 12.9% of final body weight (about 223 mg). The actual amount of water loss, however, is probably lower than 28.8 mg if one considers that the R.H. next to the host body is probably higher than 0% and that rate of water loss may decrease with time.

DISCUSSION

The critical temperature (CT) of the ticks changed several times during their development from engorged nymph to engorged adult. It was low in engorged nymphs 1 day after drop-off, increased during the following 15 days, decreased slightly just after ecdysis, remained constant in unfed adults for the next 2 months and then decreased again during engorgement of adult females. Three biological functions of such changes have been postulated.

(1) Lees (1947) suggested that CT in ticks is correlated with resistance to desiccation. Therefore the higher the CT (often above lethal temperature in argasid ticks) the greater the ability to resist desiccation at temperatures within the biological range. Although this may be true, in general, when comparing different species of ticks, it is apparently not consistent with the facts in the case of *H. leporispalustris* when comparing different developmental stages within the species. For example, in this species, engorged females have a lower CT and also a lower rate of water loss at 25 °C than unfed females. Therefore low rate of water loss does not necessarily accompany high CT.

(2) It has been proposed (Nelson & Camin, 1967) that low CT, in the range of host body temperature, may permit the elimination of excess water and aid in the concentration of the blood meal of a feeding tick. The calculated amount of water loss for *H. leporispalustris* at 0% R.H. and 37 °C is equivalent to approximately 12.9% of the total body weight of the engorged tick in 24 h. Because ticks on the host are usually subjected to much higher humidities and to lower temperatures, especially when attached to the ears, the actual amount of water eliminated by this route is probably negligible. Tatchell (1967) demonstrated that the cattle tick, *Boophilus microplus*, eliminates most of the excess water by injecting it back into the host by way of the saliva. Studies of the changes in ultrastructure of the salivary glands of *H. leporispalustris* during feeding (Kirkland, 1971) indicate that the same mechanism may be operative in the rabbit tick.

(3) It has been postulated that evaporative water loss, because of a CT lower than the body temperature of the host, might stimulate the attachment of the rabbit tick (Nelson & Camin, 1967) or the initiation of the rapid engorgement phase (J. H. Camin, personal communication) of the feeding pattern. Preliminary data indicate that CT probably does not fall within the range of host body temperature until just before the rapid engorgement phase, so CT is probably not responsible for attachment. However, the latter hypothesis is supported by a close correspondence between the host's body temperature and the CT of the ticks just prior to the rapid engorgement phase. Further support will depend on the elucidation of the sensory mechanism that might be involved in such response.

Because CT has been known to be a physico-chemical phenomenon of cuticular lipids in many arthropods (Ramsay, 1935; Wigglesworth, 1945; Beament, 1964), it is likely that changes in CT during different developmental stages are related to changes in cuticular lipids. Such a mechanism of CT will be presented in the next report (Davis, 1973a).

SUMMARY

1. During the developmental period from nymphal to adult engorgement of the rabbit tick, *Haemaphysalis leporispalustris*, critical temperature changed with developmental stage and nutritional state, but was similar in the two sexes.

2. Critical temperature was low in engorged nymphs one day after drop-off (in the range of 36–40 °C), increased during the following 15 days (46–47 °C), slightly decreased just after ecdysis (42–43 °C), remained constant in unfed females for the next 2 months (42–43 °C) and then decreased again during engorgement of adult females (from 42–43 to 37–38 °C then to 35–36 °C). The rates of water loss also changed with the developmental period.

3. The hypothesis that evaporative water loss might initiate the rapid engorgement phase is supported, although not confirmed, by a close correspondence between the host body temperature and the critical temperature of the tick just prior to the rapid engorgement phase.

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