CHAPTER 5

Temperature tolerance.
Temperature tolerance

The temperature effects on an animal may be mirrored in its physiological activities (Dean and Vernberg, 1965). Therefore the basic knowledge about the temperature relations of an animal is vital before dealing with the physiology and ecology of its thermal tolerance. Thermal tolerance of the teleost was studied by Brett (1956), Fry (1958, 1967), Fisher (1958), Hoar and Robertson (1959), Weatherley (1963a, 1963b).

Temperature tolerance is chiefly influenced by physiological processes which are in turn controlled by ambient temperature. Much study has been done on relationship between lethal temperature and acclimation temperature (Brett, 1956; Fry, 1967). The invertebrates become increasingly tolerant to heat as it is adapted to higher temperature within the range of thermal tolerance of its species and that its acclimatization to low temperature ensures a loss of heat tolerance (Grant, 1955; Spoor, 1955; Farmanfarmaian and Giese, 1963; Edney, 1964; Read, 1967; Nagabhushanam and Kulkarni, 1970; Nagabhushanam and Lomte, 1970; Staszak and Mutchmor, 1973a).

Bovbjerg (1952) showed that two species of crayfish, Orconectes propinquus and Cambarus fodiens, became increasingly tolerant to temperature between 34°C and 35°C as advancing season warmed their habitats or after they had been maintained in warm water in the laboratory for five to six weeks.
Edney (1964) has demonstrated that acclimation to high temperature while raising upper lethal limit, also raises the lower lethal limit and vice-versa in the terrestrial isopod, *Procellio laevis* and *Armadillidium vulgare*. Chill-coma temperature of an insect has been proved to be dependent on the immediate thermal history of the animal (Mutchmor and Richards, 1961). Grant (1955) had demonstrated that the heat tolerance of the earthworm, Pheretima hupeiensis increases 0.3° per 1°C rise in conditioning temperature.

When an animal is exposed to environmental conditions differing from those in which it lives, physiological adjustments or acclimatization may ensue (Vernberg and Vernberg, 1970). The concept, that poikilotherms regulate the metabolism and activity in compensatory direction against the ambient thermal variations is founded on the data concerning different poikilothermic vertebrates and invertebrates from thermal regimes such as warm and cold in the laboratory (McWhinnie and O'Connor, 1967).

The displacement of the rate-temperature (R-T) curves on acclimating the animal to the warm and cold is much more widely used criterion for assessing the temperature compensation in poikilotherms which reflect not only the mere presence or absence of compensation, but also the nature of
underlying biochemical events at the cellular level leading to the compensation (Prosser, 1958).

As no study has been done on temperature tolerance in ticks, the present work was undertaken to study the effect of various temperatures on the *Hyalomma anatolicum anatolicum* along with the changes in biochemical constituents, following thermal acclimation.

**Materials and Methods**

Adult ticks were from laboratory bred population and were maintained in glass vials. The ticks were conditioned to total darkness to eliminate the possible photoperiodic influences. The ambient temperature of the ticks fluctuated between 25°C to 28°C. During the entire period of investigation no special feeding allowance was permitted to the animals. The ticks were maintained at 75 to 80% R.H. For acclimation, the groups of 20 to 40 ticks were selected and placed in small tubes kept inside the incubators at 38.0°C ± 0.5°C (warm acclimated) or 18.0°C ± 0.5°C (cold acclimated), for different time intervals.

Initially, the animals were brought to the desired acclimation temperature by adopting 'slow induction of thermal stress pattern'.

Lethal temperatures of experimental and control animals were ascertained by exposing them to 14°C, 16°C, 18°C, 38°C, 40°C and 42°C for 24 hours (except otherwise specifically cited).
The ticks were tested in groups of ten, in small glass tubes, the temperature of which was controlled to 0.1°C. Two hours period was allowed for the ticks to equilibrate with test temperature. After the exposure to the test temperature for the desired period, the animals were classified as dead since there was no visual response to the mechanical stimulations.

Compensatory changes in water, glycogen, fat and protein accompanying the thermal acclimation were determined from the whole body of the group of ten ticks after 15 days. Water, fat, carbohydrate and proteins were measured according to the methods mentioned in Chapter on biochemical constituents.

Results

A. Heat tolerance of normal ticks.

Normal ticks conditioned in the laboratory for a week at a temperature which usually ranged between 25°C - 28°C, when exposed to 38°C, ticks displayed surviving potential for an indefinite period. At 40°C they could extend their life span maximally for 6 days whereas none of them could sustain a temperature of 42°C for 24 hours. Their percent mortalities after 12 and 24 hours are shown in Fig.1. The 12 hour median heat tolerance (or LT-50 after 12 hours) as emerging from Fig.1 was in the proximity of 40.8°C and that for 24 hours 40.5°C. These two values do not present a striking difference.
Fig. 1

Percentage mortality of
Hyalomma anatolicum anatolicum
after 12 and 24 hours at
different test temperatures.
B. **Loss of heat tolerance.**

Acclimation to cold (18.0° ± 0.5°C) sharply curtailed a characteristic 24 hour median heat tolerating capacity of the normal ticks and ten days after the acclimation it dropped to 37.5°C. Beyond this period (after 12 days) the value became stabilized at 37.4°C; with no further decline even after 3 months (cited as final temperature in Fig.3).

C. **Gain of heat tolerance.**

Heat tolerance of normal ticks which was eclipsed during cold acclimation was reclaimed quite expeditiously when the ticks were withdrawn from the cold acclimation. Twenty ticks which were maintained at 18.0° ± 0.5°C for two weeks, a period which would have ensued a loss of about 3°C in their initial heat tolerance capacity and would have reached a 24 hour median heat tolerance of about 36.5°C (Fig.3), were warmed slowly upto the laboratory temperature (25°C) over a span of about three hours and then they were kept at this temperature for twelve hours. When these animals were exposed to 40°C they exhibited 20% mortality after twelve hours and 40% mortality after 24 hours. Obviously their heat tolerating capacity was well within the frontiers of heat tolerance of normal ticks. The rate of gain of heat tolerance is conspicuously quicker than the rate of loss, the tolerance lost during two weeks at 18.0° ± 0.5°C being regained in not more than twenty four hours.
Fig. 2

Loss of cold tolerance,
gain of cold tolerance, and
effect of cold acclimation
(18.0° ± 0.5°C) in
Hyalomma anatolicum anatolicum
D. **Effect of warm (38.0° ± 0.5°C) acclimation on the heat tolerance.**

Warm acclimation augmented the heat tolerance of normal ticks by about 2°C. After ten days and with the elapse of another two days their 24 hour median heat tolerance was found to be stationed at 42.5°C, which did not increase even three months (cited as final temperature in Fig.3) after the acclimation.

E. **Cold-tolerance of normal ticks.**

Normal ticks (maintained at 25° - 28°C for a week) were able to endure 18°C temperature indefinitely. At 16°C they lived for five days but none of them could tolerate the temperature of 14°C for even twenty four hours. The mortalities that occurred at these three temperatures are presented in Fig.4. The 12 hour median cold tolerance as seen from Fig.4 is 15°C and that for 24 hour is 15.6°C. From the above data conclusion can be drawn that the ticks are more susceptible to cold rather than heat since its 24 hour median cold tolerance is about 9°C, below the conditioning temperature where as its heat tolerating capacity is about 14°C ahead of its normal thermal regime.

F. **Loss of cold-tolerance.**

Normal individuals lost their peculiar cold tolerating ability when they were acclimated to warm (38.0° ± 0.5°C) and incidentally there was no latent period before the commencement
Fig. 3
Loss of heat tolerance (18.0°C ± 0.5°C), gain of heat tolerance (percentage mortality) after 12 and 24 hours and effect of warm acclimation (38.0°C ± 0.5°C) in *Hyalomma anatolicum anatolicum*. 
of the decline in cold-tolerance. There was a fall by about 6°C in the 24 hour median cold tolerance of the normal ticks from 16.0°C - 22.6°C after twelve days (Fig.2) of warm acclimation.

G. **Gain of cold-tolerance.**

The cold-tolerance deprived during warm acclimation was restored quite urgently when the ticks were shifted out of the warm acclimating vessels. Twenty ticks conditioned at 38.0°C ± 0.5°C for two weeks (during which their 24 hour cold tolerance would have decreased to 22.6°C, Fig.2) were slowly cooled down to the laboratory temperature for twelve hours. When these ticks were exposed at 16.0°C ± 0.5°C, 40% and 60% mortality was recorded after 12 and 24 hours respectively.

H. **Effect of cold acclimation (18.0°C ± 0.5°C) on cold tolerance.**

When normal individuals were acclimated to cold (18.0°C ± 0.5°C) their cold tolerating capacity was raised by nearly 2°C (Fig.2), at the end of twelve days. However any further continuous acclimation (even 3 months period cited as final temperature in Fig.2) did not advance their 24 hour cold tolerance below 13.5°C.

*Biochemical changes following acclimation.*

The data showing the changes in biochemical constituents
Fig. 4

Percentage mortality of
Hyalomma anatolicum anatolicum
at different test temperatures
( cold tolerance )
Table 1. Changes in different chemical constituents of *Hyalomma anatolicum* anatolicum acclimated to warm (38.0 ± 0.5^°^C) and cold (18.0 ± 0.5^°^C) for fifteen days.

<table>
<thead>
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<th>Constituents</th>
<th>Warm acclimated</th>
<th>Normal</th>
<th>Cold acclimated</th>
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<tr>
<td>Glycogen</td>
<td>8.65 ± 1.5</td>
<td>6.4 ± 0.55</td>
<td>2.85 ± 0.55</td>
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<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
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<tr>
<td>Fat</td>
<td>13.6 ± 1.5</td>
<td>8.3 ± 1.5</td>
<td>7.1 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>Protein</td>
<td>28.33 ± 8.2</td>
<td>40.5 ± 3.7</td>
<td>46.55 ± 3.25</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>Water %</td>
<td>70.05 ± 7.2</td>
<td>66.0 ± 5.5</td>
<td>62.02 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
</tbody>
</table>

a) Values of glycogen, fat and protein are expressed as mg % ± S.D. of dry wt.

b) Number in parenthesis indicates number of observations.
like body glycogen, fat, protein and water of the tick following acclimation to warm (38.0° ± 0.5°C) and cold (18.0° ± 0.5°C) for fifteen days are shown in Table 1. The percentage of glycogen and water showed significant decrease with the decrease in the conditioning thermal values. The percentage of body fat registered a decline with the lowering of acclimating temperature (from 13.6% at 38.0°C to 7.1% at 19.0°C).

The compensatory fluxes in body protein are somewhat comparable with that of glycogen. Warm acclimation retarded the protein content whereas rise in its value was recorded following the cold acclimation from the normal body protein percentage. Body water also showed increase in its percentage on warm acclimation.

**Discussion**

The literature on thermal relations of poikilotherms singularly signalises that lethal temperature of a species is not an absolute entity but is anchored to the immediate thermal history of each particular organism. Consequently, the figures for median heat and cold tolerance are not significant unless acclimation temperature is also cited. Moreover, acclimation influences not only the upper and lower incipient lethal temperatures, but also the duration of exposure to a lethal temperature that an animal can withstand (Andrewartha and Birch 1954; McWhinnie, 1967). The same trend is reflected
through the data obtained in the present investigation on the tick *Hyalomma anatolicum anatolicum*. Since there is an acute shortage of data based on competent, standardized or same conditioning thermal regimes for acclimation the effort of bringing out the salient features of temperature tolerance of different invertebrates is not rewarding.

The results of present investigations lead to the conclusion that *H. a. anatolicum* exhibits a simple pattern of acclimation effects in relation to lethal temperatures (median heat and cold-tolerance). Decline in heat and cold tolerance is appreciably greater following cold and warm acclimation respectively as opposed to the increase in temperature tolerating capacities after thermal acclimation. The effect of high temperature raises both upper (both physiological and numerical increase) and lower (only numerical increase) lethal temperatures, whereas low temperature lowers both (only numerical decline in case of cold tolerance). Fully acclimated state (high or low temperature) seems to be approached asymptotically (as in isopod, *Porcellio laevis* and *Armadillidium vulgare*, Edney, 1964) and achieved in about 10 to 12 days.

The slug, *Laevicaulis alplex* possessed the 24 hour median heat tolerance of 34.5°C which was conditioned at 25-30°C (Nagabhushanam and Kulkarni, 1970). Edney (1964)
has recorded the upper lethal temperature (after 30 minutes exposure) of about 38.5°C for *Porcellio laevis* and 39.5°C for *Armadillidium vulgare* maintained at 20°C.

In the present study probably after 12 days (acclimation being presumably completed) genetic forces are marshalled out which thwart any further attempts to cold acclimation in subsequent slashing down of the heat tolerance limit. It is only after three days that 24 hour median heat tolerance of normal ticks deviated markedly from original value. It shows similarity with other poikilotherms like minnow, *Pimephales promelas* (Brett, 1944) and crayfish, *Orconectes rusticus* (Spoor, 1955). In this context 24 hour median heat tolerance of 39.5°C of *H. a. anatolicum* shows its capability to sustain higher temperature for longer periods.

Possibly 42.5°C represent maximal heat tolerance capacity of the ticks under experimentation and any subsequent elevation in this temperature is most probably beyond the pale of phenotypic manipulations (which were employed in the current experiments) as that may evoke the genetic tamperings in the animals (Vernberg, 1963).

On the other hand 24 hour cold-tolerance of *H. a. anatolicum* which is 16.3°C, shows its poor ability to endure the cold as compared to other terrestrial invertebrates. The rate of gain of cold-tolerance is exceedingly less than the rate of gain of heat tolerance. This may be an ecological
prerequisite of the habitat's in tropical terrain.

As demonstrated by Brett (1944) in certain fishes and Hutchinson (1961) in salamanders, *Hyalomma* shows a faster rate of gain of heat tolerance than that of cold tolerance. These two factors (greater heat tolerating capacity and more rapid gain of heat tolerance) are probably adaptively significant and might have been spurred by ecological necessity of its tropical habitat.

An increment in acclimation temperature (within the genetic limit of species) can elevate the median heat tolerance whereas acclimation to low temperature entails a loss in the heat tolerating ability (Doudoroff, 1942; Brett, 1944, 1946; Edney, 1964; Nagabhushanam and Lomte, 1970). The present probe reveal that the tick, *H. a. anatolicum* is no exception to the findings of earlier workers as its 24 hour median heat tolerance is elevated by 2°C after twelve days of acclimation at 18.0°C ± 0.5°C from normal value of 39.5°C. Similarly acclimation to cold (18.0°C ± 0.5°C) for twelve days brings a down fall in its heat tolerance by 3.0°C. Drastic decrease by 6°C, in the 24 hour median heat tolerance of the normal individual, is observed following the acclimation to warm (38.0°C ± 0.5°C) for twelve days whereas increase (physiological) in the cold tolerance of normal individuals by about 2°C is induced by the cold (18.0°C ± 0.5°C) acclimation for twelve days.
Acclimation effects of the similar order as the present one have been transmitted by various other groups; e.g. fish *Carassius auratus* (Brett, 1946), crayfish, *Orconectes rusticus* (Spoor, 1955), isopods, *Porcellio laevis* and *Armadillidium vulgare* (Edney, 1964), fresh water mussel, *Parreysia corruqate* (Nagabhushanam and Lomte, 1970).

Though there exists a quantitative variation in the extent and rate of acclimation, qualitatively there is a coherent, fundamental unity of design of temperature acclimation among the different animal groups (Spoor, 1955, Nagabhushanam and Kulkarni, 1970). Alterations in heat and cold-tolerance limits in response to the acclimation indicate that in ticks there can be a substantial anticipatory adjustment in their lethal temperatures (temperature tolerating abilities) as has been hinted for other poikilothersms by Fry and Hochachka (1970).

It is known that two types of processes often occur during acclimation; (a) compensatory adjustment in metabolic rate, which tends to free the organism from the stringencies of external environment and (b) biophysical and biochemical restructuring of many cellular and tissue components for operating under the new thermal regimes imposed on the organism (Fry and Hochachka, 1970). The existence in poikilothersms of metabolic compensation for thermal stress is undisputed (Parvatheshwararao, 1967). The physiological compensation is
the principal homeostatic device exploited in order to function in a habitat of oscillating thermal conditions (Fry and Hochachka, 1970; Vernberg and Vernberg, 1970; Parvatheswararao, 1972).

Recently, compensatory changes in carbohydrates, proteins, fats, electrolytes and enzymes have been elaborately worked out in certain poikilotherms (Hoar and Cottle, 1952; Rao, 1961; Johnston and Roots, 1964; Dean and Vernberg, 1965; Parvatheswararao, 1967; Das and Krishnamoorthy, 1969; Newell and Pye, 1971; Umminger, 1971. Saroja and Rao (1965) studied the compensatory changes in inorganic ions, glycogen, RNA, protein, and water content of the tissues following thermal acclimation in Lampito mauritii. Nayeemunnisa and Rao (1972) in L. mauritii observed the compensatory fluxes in liquid metabolism in response to temperature acclimation. They showed that total fat content, formic and oxaloacetic acid levels declined significantly on acclimation to decreasing temperature, whereas unsaturation of lipids, the lipase activity, the levels of acetate, B-hydroxybutyric and acetoacetate acids and cholesterol showed a significant increase on cold acclimation.

In many cases (Hoar and Cottle, 1952; Suhrmann, 1955; Kanungo and Prosser, 1959), including the present, body water exhibits a direct proportionality with the acclimation temperature. Elevation in body water at higher temperature
may be because, water enters more rapidly at higher temperature than at lower temperature owing to the increased permeability and diffusion of the cell membrane (Hoar and Cottle, 1952). Such fluxes might mean concomitant changes at the cellular level, perhaps involving enzyme activity, (Kanungo and Prosser, 1959). The increased water during warm acclimation may slash the metabolic rate by reducing the proportion of respiring tissue (Parvatheswararao, 1967). The opposite would be then true on cold acclimation when water content declines. This may be happening in *H. a. anatolicum*.

Parvatheswararao (1967) has evidenced the increased amino acid incorporation into cellular proteins on cold acclimation in fish, *Etropus maculatus*. Hence, it can be concluded that some of the metabolizing protein constituents might be shunted into the fat synthesizing pathways during warm acclimation of *H. a. anatolicum*.

The present observations indicate that animals are resorting to proteolysis on warm acclimation for satisfying energy demand and also for augmenting lipid and glycogen stores. The data further indicate that these ticks might be mobilizing lipids and glycogen for physiological activity and for increasing protein level during cold acclimation.

The present study on *H. a. anatolicum* show increase
Summary

Hyalomma survived maximally for 6 days at 40°C and 24 hours at 42°C. The median heat tolerance after 12 hours was 40.6°C and 40.5°C after 24 hours.

Acclimation to cold (18.0°C ± 0.5°C) curtailed the median heat tolerating capacity in normal ticks and 10 days after the acclimation the value stabilized at 37.4°C with no further decline even after 3 months.

When ticks maintained at 18.0°C ± 0.5°C for two weeks were exposed to 40°C they showed 20% mortality after 12 hours and 40% after 24 hours. The rate of gain of heat tolerance was greater than rate of loss.

In warm acclimation (38.0°C ± 0.5°C) median heat tolerance was found to be 42.5°C which was constant even after three months. The twelve hour median cold tolerance was found to be 15°C and for 24 hours it was 15.6°C.

There was a fall by 6°C in 24 hours median cold tolerance of the normal ticks.

The cold tolerating capacity was raised by 2°C when normal ticks were acclimated to cold (18.0°C ± 0.5°C).

The glycogen and fat levels increased on warm acclimation. On cold acclimation glycogen was found to be decreased as well as fat. On the other hand the protein content increased during cold acclimation and fell on warm acclimation. Water content was more on warm acclimation and less under cold acclimation.
References


Brett, R.J. 1956 Some principals in the thermal requirements of fishes. Q. Rev. Biol; 31: 75-87.


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<th>Author(s)</th>
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