

SALT AND WATER BALANCE IN ARTHROPODS: THE EFFECTS
OF DESICCATION AND REHYDRATION ON THE LONE
STAR TICK, AMBLYOMMA AMERICANUM (LINNAEUS)

By

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PREFACE

This study was concerned with the effects of desiccation and rehydration on the haemolymph concentrations of sodium, potassium, calcium and magnesium, haemolymph volume, osmolarity and total body water content of the lone star tick, and to determine if the tick has abilities to regulate any of these constituents.

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TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION	1
II. REVIEW OF THE LITERATURE	3
III. MATERIALS AND METHODS	10
Experimental Animals	10
Determinations of Gains and Losses of Water	10
Method of Comparing Data	11
Determining Concentrations of Ions	12
Blood Volume	12
Freezing Point Depression	12
Water Content	13
IV. RESULTS	14
Water Content Percent	14
Blood Volume	17
Haemolymph Osmolarity	20
Potassium Concentration	22
Sodium Concentration	22
Magnesium Concentration	22
Calcium Concentration	26
V. DISCUSSION	28
VI. SUMMARY AND CONCLUSIONS	34
LITERATURE CITED	36

LIST OF TABLES

Table	Page
I. Water Content Percent of "Moderately Hydrated", "Severely Desiccated", and "Rehydrated" Ticks	15
II. The Effects of Desiccation and Rehydration on Whole Body Absolute Units of Water	16
III. Haemolymph Volume Percent of "Moderately Hydrated", "Severely Desiccated", and "Rehydrated" Ticks	18
IV. The Effects of Desiccation and Rehydration on Absolute Haemolymph Volume Percent Units	19
V. Haemolymph Freezing Point Depression ($-\Delta^{\circ}\text{C}$) of "Moderately Hydrated", "Severely Desiccated" and "Rehydrated" Ticks	21
VI. Haemolymph Concentration of Potassium of "Moderately Hydrated", "Severely Desiccated", and "Rehydrated" Ticks	23
VII. Haemolymph Concentration of Sodium of "Moderately Hydrated", "Severely Desiccated", and "Rehydrated" Ticks	24
VIII. Haemolymph Concentration of Magnesium of "Moderately Hydrated", "Severely Desiccated", and "Rehydrated" Ticks	25
IX. Haemolymph Concentration of Calcium of "Moderately Hydrated", "Severely Desiccated", and "Rehydrated" Ticks	27

LIST OF FIGURE

Figure	Page
1. Combined Effects of Desiccation and Rehydration in the Lone Star Tick. The first set of three bars indicates the whole body water content percent. All other sets signify the indicated property of the haemolymph.	31

CHAPTER I

INTRODUCTION

An essential aspect of the survival of an animal is determined by its ability to tolerate or regulate the level of its internal constituents. The activities of cells and tissues are most efficiently carried out within a narrow range of osmotic pressures.

Most of the life cycle of the lone star tick, Amblyomma americanum (L.), is spent between blood meals with only a small fraction occurring while the parasite is feeding and living on the host (Hair and Howell, 1970). High numbers of this acarine are distributed in the Ozark mountain region, an area characterized by dense underbrush. This environment provides a microclimate in which the humidity remains high throughout the year (Semtner, et. al., 1971).

Investigations have shown that some arthropods, including the lone star tick (Sauer and Hair, 1971), have the ability to absorb water from atmospheres of high relative humidity (Edney, 1966; Lees, 1946; Hafez, et. al., 1970; Noble-Nesbitt, 1970; Wharton and Kanungo, 1962; Browning, 1954). It is generally believed that desiccated ticks gain weight by absorbing water vapor from the air into the haemolymph (Hafez, et. al., 1970). A gain or loss of body water raises the question of what effect these phenomena have on the nature of the haemolymph constituents. The necessity for optimum levels of solutes and ions on other physiological

processes like nerve and muscle are well-known (Stobbart and Shaw, 1963; Treherne, 1965).

The aim of the present study was to measure the effects of the absorption and loss of water by the lone star tick on the haemolymph concentrations of sodium, potassium, calcium, magnesium, haemolymph volume percent, osmolarity and total body water content and to determine if the organism has abilities to regulate any of these constituents.

CHAPTER II

REVIEW OF THE LITERATURE

The lone star tick, Amblyomma americanum (L.) is an economically important pest in the Ozark region. It depends on three separate blood meals to complete its life cycle. Probably all species of vertebrate animals, higher than fishes, are subject to attack by ticks, but particularly mammals, including wild and domestic animals and man, with warm blood are highly attractive to them (Herms and James, 1961). For sucking blood, both the hypostome and the chelicerae of the mouth parts are inserted into the skin of the host and numerous diseases are then transmitted by this pest to the host, among which are Rocky Mountain spotted fever, tularemia, Q fever, lone star virus, Bullis fever and tick paralysis. The length of time that ticks remain attached depends on the stage of development, but only a small fraction of the life cycle is actually spent on the host; the remaining period of time is spent on the ground (Hair and Howell, 1970).

Numerous investigators had shown that development, activity and the survival of ticks are influenced greatly by the temperature and humidity within the tick's microhabitat. Feldman-Muhsam (1951) found that low temperature and high humidity were most favorable to the survival of ticks; for example, at 17.5°C and 80 to 95 percent relative humidity, the mean longevity of Hyalomma savignyi is about one year. The same worker (Feldman-Muhsam, 1947) found that larvae and nymphs

are more sensitive to humidity than to temperature. Lees (1946) has shown that the sheep tick (Ixodes ricinus) lost fifty percent of its original weight within 24 hours if kept in a container of zero percent relative humidity and lost five percent at seventy percent relative humidity. Lancaster and McMillan (1955) indicated that lack of moisture reduced the survival of the lone star tick. Recent ecological studies by Semtner, et. al. (1971) have placed further emphasis on the correlation between the lone star tick's distribution and the type of vegetation in which they live. These workers found that high numbers of ticks were found in dense ground coverage in which the humidity remains high throughout the year.

Most of the desert-inhabiting arthropods have made many adaptations for the purpose of conserving water. They may have a relatively impermeable cuticle, internal respiratory surfaces, and relatively insoluble excretory products. Besides, many have a mechanism to gain water from subsaturated air, through drinking, utilization of metabolic water, absorption from food or the absorption of water in contact with the cuticle. In ticks and other relatively small terrestrial arthropods, because of their relatively large exposed evaporating surface, their ability to survive in dry conditions is due to their success in reducing the loss of water through the cuticle and or spiracles and their ability to absorb water vapor from unsaturated air (Hafez, et. al., 1970).

The above absorption phenomena has been demonstrated in several arthropods: In ticks by Lees (1946), Browning (1954), Hafez, et. al. (1970) and Sauer and Hair (1971), and in mites by Wharton and Kanungo (1962), and in the insects Thermobia (Noble-Nesbitt, 1970; Okasha, 1971)

and Arenivaga (Edney, 1966). It is generally believed that weight changes in arthropods when subjected to high or low relative humidity closely approximate gains or losses of water content (Wharton and Kanungo, 1962; Edney, 1966; Hafez, et. al., 1970; Sauer and Hair, 1971).

There is evidence that arthropods have a metabolically controlled spiracular closing mechanism (Hefnaway, 1970; Browning, 1954; Mellanby, 1935; Loveridge, 1968), and secrete impermeable wax over the epicuticular surface to reduce water loss (Beament, 1944; 1948; 1955; 1961; Wigglesworth, 1945; 1948; Lees, 1947; 1948; Schulman, 1948; Mead-Briggs, 1956; Locke, 1965; Beament, et. al., 1964; Hafez, et. al., 1970). While the mechanism for absorbing water vapor from subsaturated atmospheres is still obscure, the site of water exchange with the atmosphere is varied.

In most arthropods, the general water-reservoir is the blood, and most changes in the water content of the animal are accommodated for by changes in the volume of the blood. Therefore, the water which is taken up from the atmosphere may eventually go into the haemolymph. Both the blood and the cell of most animals are isosmotic with a one percent sodium chloride solution equivalent to ninety-nine percent relative humidity, while the humidities that animals can take up water from the atmosphere is species specific but may be as low as forty-five percent relative humidity in Thermobia (firebrat) (Beament, et. al., 1964). Thus water passes from air into the blood against the concentration gradient. Wharton and Kanungo (1962) also showed that the transpiration rates of live and dead mites are different. In addition, dead mites at hydrating relative humidity lose rather than gain weight, indicating that the absorption process occurs only in live animals.

Okasha (1971) pointed out that the water absorption mechanism is concerned with volume regulation. Browning (1954) assumed that this mechanism in ticks was mainly through the activity of the epidermal cells because the absorption mechanism was inhibited when ticks were subject to high concentration of CO₂ (30 to 45 percent). Lees (1946) found that the exchange of water of ticks took place mainly through the cuticle and was regulated by the secretory activity of the epidermal cells. Beament (1965) noticed that while water moved across a layer of lipid, the lipid acted as a semi-permeable membrane and the active uptake of water could not occur if this lipid was grossly disrupted. His conclusion was that water was actively absorbed through the cuticle into the haemolymph.

Insect haemolymph is composed of haemocytes and plasma and the blood plasma consists mainly of water and soluble components. It serves as a bathing medium for the cells of most tissue. The composition and the osmolarity of the haemolymph are species specific. The differences are substantial not only between species, but between different stages in the life history of a single species (Lee, 1961; Brady, 1968; Wharton, *et. al.*, 1965; Buck, 1953; Pichon, 1970; Wall, 1970; Stobbart and Shaw, 1963; Hoyle, 1954; Croghan and Lochwood, 1960). The activities of cells and tissues are most efficiently carried out within a narrow range of osmotic pressures. It would seem that for a given species of insect, at a given stage in its life history, there is a general level of osmolarity and a proper proportion of soluble components for normal physiological function. Changes in the total body water content will affect the haemolymph volume which result in

changes in the concentration of the haemolymph which may eventually affect nervous and muscular activity (Treherne, 1965; Hoyle, 1954).

It is well known that arthropods have the ability to tolerate or regulate their haemolymph volume and constituents under different environmental conditions such as desiccation, hydration, feeding, and starvation, by moving of salts and water and changing the relative amounts of soluble protein and amino acid in the haemolymph (Djajakusumah and Miles, 1966; Wall, 1970; Pichon, 1970; Beadle and Shaw, 1950; Sutcliffe, 1961; Mellanby, 1939; Buck, 1953; Lee, 1961). In most insects the burden of regulation falls largely on the excretory system by producing urine in the Malpighian tubules and selective reabsorption of constituents from the rectal glands of the hindgut.

The Malpighian tubules lie free in the haemolymph of the abdomen; they are closed distally and open proximally to the alimentary canal at the junction of midgut and hindgut. There is some histological differentiation between tubules, or between different regions of a single tubule. In Dixippus (Ramsay, 1955b) there are some gradations of physiological activity along the length of the superior tubules; Na, K, and water are secreted into the tubule at all levels, but the Na/K ratio is greater in the proximal region. The proximal and middle regions of the inferior tubule are similar to the superior tubules, but the distal portions produce little secretion. The Malpighian tubules in Rhodnius (Wigglesworth, 1931b; 1931c) are only of one kind, and secretion occurs only in the distal region; the proximal region is involved in reabsorption rather than secretion.

Urine secretion occurs as a consequence of transporting potassium from the blood into the lumen of the Malpighian tubules against the

concentration gradient (Ramsay, 1953; 1954; 1955b; Irvine, 1969; Berridge, 1968). Berridge (1968) suggested that the secretion of potassium was achieved by two pumps working in a series: a coupled sodium-potassium exchange pump on the basal membrane and an electrogenic pump on the apical surface. Anions, water, and metabolically useful substances such as amino acids, sugars, and urea were thought to flow passively down the osmotic gradients created by the active secretion of potassium (Ramsay, 1958; Berridge, 1968). The submicroscopic structure of the membrane of the Malpighian tubule shows that the inner surface is extensively folded to produce a layer of tightly packed microvilli, the basal side is marked by extensive invagination of the plasma membrane into the cell. Mitochondria are closely associated with both membranes suggesting a source for energy for the active transport of potassium and sodium alluded to above. Such structures provide a large surface area to establish the standing osmotic gradients for coupling of water and solute transport, as described for the transport of water across other epithelial membranes (Diamond and Bossert, 1967; 1968; Berridge, 1968; Diamond and Tormey, 1966; Tormey and Diamond, 1967). The rate of secretion of ions is approximately proportional to the concentration of the ions in the haemolymph (Ramsay, 1955b) and it is also controlled by a diuretic hormone (Maddrell, 1963; Pilcher, 1970). Therefore, the Malpighian tubules have the ability to maintain the normal composition of the haemolymph.

The contents of Malpighian tubules enter the alimentary canal to mix with end products of digestion from the midgut and pass through the hindgut to the rectum. The rectal gland and epithelium of the

hindgut reabsorb the wanted salts and water and play the major role in regulation.

Phillips (1964) noticed that in starved locusts supplied with tap water, more salt than water was reabsorbed, while those supplied with hypertonic saline, the rectum was distended with fluid and water was reabsorbed. He pointed out that the absorption of salts was an active process and water was also actively absorbed against a concentration gradient without a net uptake of inorganic ions. Berridge, *et. al.* (1967) and Oschman, *et. al.* (1969), however, from the submicroscopic level concluded that solute coupled water transport is the possible mechanism responsible for the water reabsorbed across the rectal epithelium. The rate of rectal water absorption may be controlled by an antidiuretic hormone (Mills, 1967; Wall, 1967).

Blood sucking arthropods, which ingest large volumes of blood during feeding, regulate their internal concentration by the elimination of excess water. This water may be eliminated by the Malpighian tubules of mosquitoes, tsetse flies and bugs. In ticks, however, the Malpighian tubules perform an excretory function; excess water is eliminated by the coxal gland of argasid ticks (Lees, 1946b), or via the salivary glands in hard ticks (Tatchell, 1967; 1969). The precise mechanisms of water and ion regulation by feeding ticks is still obscure.

CHAPTER III

MATERIALS AND METHODS

Experimental Animals

Animals used in all experiments were unfed adult lone star ticks, Amblyomma americanum (L.). Because of the large numbers required, both field collected (from May, 1971 to September, 1971) and laboratory reared (one to six months old) ticks were used. Animals obtained from the laboratory were fed on rabbits and were continuously held in high relative humidity (R.H.) chambers (80 to 90 percent) prior to experimentation. Ticks collected in the field were held in similar high humidity chambers for at least ten days before experimentation.

Determinations of Gains and Losses of Water

The gains and losses of weight of desiccated ticks (after brief exposure to hydrating or dehydrating humidities) reflect gains and losses of water (Sauer and Hair, 1971). Therefore, to obtain ticks in which a gain or loss of water preceded measurement of the haemolymph properties, the methods of these authors were followed. Specifically, groups of unfed adults (five to ten per group) were subjected to twenty-four hours of predesiccation at zero percent R.H. After this preliminary pre-conditioning step, the ticks were weighed and placed in environments of either 98 percent R.H. or low humidity (0 percent or 42 percent R.H.) for variable amounts of time. Following these

procedures, ticks were weighed once more to obtain a measure of the gain or loss of water. To obtain ticks over a full range of desiccating and hydrating conditions, it was necessary to measure the maximum weight losses possible (without causing high mortality) and maximum possible weight increases after exposure to high humidity. The maximum losses without causing high mortality of pre-desiccated ticks was about -20 percent. Likewise, the maximum increases for similar ticks was about +25 percent. The maximum increases were achieved within two to four days after introduction of the animals to the high humidity.

Method of Comparing Data

To facilitate comparisons of the various haemolymph constituents, we decided to place our results into groups according to the tick's prior change in weight. The first category included the results from ticks that gained or lost no more than five percent of their post-desiccation weight (-5 to +5 percent). Ticks that underwent this level of weight change are referred to as "moderately hydrated" in the results and discussion that follow (Tables I to IX). Results from animals that lost at least ten percent of their post-desiccation weight (i.e., "severely desiccated" ticks) were placed into a second group and results from ticks that increased at least ten percent above their post-desiccation weight ("rehydrated" or "fully hydrated" ticks) following exposure to rehydrating conditions were placed into the third group. Results from ticks that gained or lost five to ten percent were not included. We were unable to show any significant differences in results when comparing males and females except for haemolymph volume

which was less in the smaller males. However, conversion of volumes to haemolymph volumes percents (ul/mg x 100) yielded little difference between the two sexes.

Determining Concentrations of Ions

Legs of experimental ticks from a batch of ticks having undergone a known weight change were cut and haemolymph was collected and pooled with finely drawn pre-calibrated glass capillary tubes (0.1 to 0.4 ul). The concentration of sodium, potassium, magnesium and calcium of the haemolymph was assayed with a Beckman 440 atomic absorption spectrophotometer after appropriate dilution with deionized water.

Blood Volume

Because of size, the method described by Richardson, et. al. (1931) was used. Groups of five ticks that had undergone known weight changes were dissected open; as much haemolymph as possible was removed (after gentle squeezing) with pieces of absorbent tissue paper. Following this operation, the ticks were weighed once more. The difference between the original weight of the ticks and the weight after removal of the blood is the approximate weight of the blood. Blood volume was estimated after the specific gravity of representative samples of tick haemolymph were measured. Since it was impossible to remove all of the haemolymph, the values are estimates.

Freezing Point Depression

The osmolarity of collected haemolymph of experimental ticks was measured with a Clifton Technical Physics Nanoliter Osmometer, expressed

as the freezing point depression ($-\Delta^{\circ}\text{C}$) having precision of $\pm 0.001^{\circ}\text{C}$. Because of the minute quantity of fluid required, only one tick was needed to supply the necessary haemolymph for each assay.

Water Content

After determining the weight changes, groups of ten ticks each were placed in a drying oven at 105°C and weighed again every eight hours until a constant weight was achieved. The difference between wet and dry weights was taken as the water content of the ticks.

CHAPTER IV

RESULTS

Water Content Percent

The water content percent of ticks undergoing slight desiccation or rehydration ("moderately hydrated") after 24 hours of pre-desiccation was 52.8 percent (Table I). "Rehydrated" ticks showed a significantly higher ($p < 0.001$) average water content percent of 60.3 while ticks that were "severely desiccated" revealed a significantly lower ($p < 0.05$) water content percent of 46.0.

To establish to what extent the above percent changes are caused by gains or losses of water from or to the atmosphere, all percentage numbers were converted to arbitrary but absolute units of weight (Table II). Ticks in which the water content was 52.8 percent ("moderately hydrated") contained 52.8 absolute weight units of water. In this scheme, the remainder, 47.2 units, are dry weight for a total weight of 100.0. There was an average of 18.2 percent more weight (18.2 units) in "rehydrated" ticks than "moderately hydrated" ticks. The water content percent of "rehydrated" ticks was 60.3 percent. Because this is 60.3 percent of a larger total weight (118.2 vs. 100.0), the absolute units of water are 71.2 for an overall increase of 18.4. This is very near the 18.2 percent more weight in "rehydrated" ticks, and therefore as expected, all the weight measured as gained during

TABLE I
 WATER CONTENT PERCENT OF "MODERATELY HYDRATED", "SEVERELY
 DESICCATED", AND "REHYDRATED" TICKS

Experimental Condition	N	% wt. Δ after Rehydration or Desiccation of Pre-desiccated Ticks	Water Content % \pm S.D.	Significance
"Moderately hydrated" ticks				
Mean	10	-1.0%	52.8% \pm 4.2	
"Rehydrated" ticks				
Mean	12	+17.2%	60.3% \pm 2.9	*p < 0.001
"Severely desiccated" ticks				**p < 0.001
Mean	4	-13.4%	46.0% \pm 4.2	*p < 0.05

*Statistical comparison of "moderately hydrated" ticks to "rehydrated" ticks and "severely desiccated" ticks (t-test)

**Statistical comparison of "rehydrated" ticks to "severely desiccated" ticks (t-test)

TABLE II
THE EFFECTS OF DESICCATION AND REHYDRATION ON
WHOLE BODY ABSOLUTE UNITS OF WATER

Experimental Condition	% wt. difference (as compared to "moderately hydrated" ticks)	Water Content %	"Absolute" H ₂ O units	"Absolute" H ₂ O units lost or gained	Wt. units lost or gained less "absolute" H ₂ O Units lost or gained
"Moderately hydrated" ticks		52.8%	52.8		
"Rehydrated" ticks	*+18.2%	60.3%	71.2	+18.4	0.2
"Severely desiccated" ticks	** -12.4%	46.0%	40.2	-12.6	0.2

*Net weight gained from -1% wt. Δ ("moderately hydrated" ticks) to +17.2% wt. Δ ("rehydrated" ticks) (Table I)

**Net weight lost from -1% wt. Δ ("moderately hydrated" ticks) to -13.4% wt. Δ ("severely desiccated" ticks) (Table I)

rehydration can be accounted for by an increase in water content. There was an average of 12.4 fewer weight units in "severely desiccated" ticks than "moderately hydrated" ticks. The water content of the "severely desiccated" ticks was 46.0 percent, but because this is 46.0 percent of a smaller total weight, the absolute units of water are 40.2 for an overall loss of 12.6 down from the 52.8 in "moderately hydrated" ticks. This is very near to the actual units of weight measured as lost, and therefore, almost all the weight lost during the experimental period of desiccation can be accounted for by a loss of water.

Blood Volume

To accurately assess the effects of desiccation and rehydration on the concentration of haemolymph solutes, it is vital to know the concurrent effects on the haemolymph volume percent. Blood volumes were estimated by the method of Richardson, et. al. (1931). Although this method lacks the precision of more sophisticated techniques such as the ¹⁴C-inulin method of Wharton, et. al. (1965), animal size precluded its use. However, because we were more interested in relative volume percentages between animals from different experimental conditions than absolute volumes, the technique proved to be adequate and precise enough as judged by standard deviations. The results are shown in Table III. In "moderately hydrated" ticks, the blood volume percent was 8.8. In "hydrated" ticks the volume percent was 22.7, an increase of 13.9 percent. Using the same reasoning as that employed in Table II for total body water, the absolute units of water in the haemolymph (Table IV) increased by 18.5 during rehydration or very near to the units of weight shown as gained by the whole tick. It is readily

TABLE III
 HAEMOLYMPH VOLUME PERCENT OF "MODERATELY HYDRATED",
 "SEVERELY DESICCATED", AND "REHYDRATED" TICKS

Experimental Condition	N	% wt. Δ after Rehydration or Desiccation	Blood volume % (μ l/mg x 100) \pm S.D.	Significance
"Moderately hydrated" ticks				
Mean	6	-1.4%	8.8% \pm 2.6	
"Rehydrated" ticks				
Mean	13	+18.8%	22.7% \pm 7.8	*p < 0.001
"Severely desiccated" ticks				
Mean	7	-11.8%	8.0% \pm 2.8	**p < 0.001 *p > 0.5

*Comparison of "moderately hydrated" ticks to "rehydrated" ticks and "moderately hydrated" ticks to "severely desiccated" ticks (t-test)

**Comparison of "rehydrated" ticks to "severely desiccated" ticks (t-test)

TABLE IV
THE EFFECTS OF DESICCATION AND REHYDRATION ON
ABSOLUTE HAEMOLYMPH VOLUME PERCENT UNITS

Experimental Condition	% Body Wt. difference (compared to "moderately hydrated" ticks)	Blood volume % (ul/mg x 100)	"Absolute" volume units in Haemolymph	"Absolute" volume units lost or gained (relative to "moderately hydrated" ticks)	Wt. units lost or gained less units lost or gained from haemolymph
"Moderately hydrated" ticks		8.8%	8.8		
"Rehydrated" ticks	*+20.2%	22.7%	27.3	+18.5	1.7
"Severely desiccated" ticks	** -10.4%	8.0%	7.1	-1.7	8.7

*Net weight gained from -1.4% wt. Δ ("moderately hydrated" ticks) to +18.8% wt. Δ ("rehydrated" ticks) (Table III)

**Net weight lost from -1.4% wt. Δ ("moderately hydrated" ticks) to -11.8% wt. Δ ("severely desiccated" ticks) (Table III)

apparent from Tables III and IV that almost all the water gained during rehydration of "moderately hydrated" ticks is taken up by the blood. After "severe desiccation", we measured a blood volume percent of 8.0 and were unable to show a significant difference between this amount and that which was present in "moderately hydrated" ticks. Since there is a significant difference ($p < 0.05$) in the total water content between "moderately hydrated" and "severely desiccated" ticks (Table I) much of the net water loss must be at the expense of other parts of the body and suggests that during "severe desiccation," the lone star tick has the ability to regulate its haemolymph volume.

Haemolymph Osmolarity

The haemolymph osmotic pressure data (measured as freezing point depression, $-\Delta^{\circ}\text{C}$) are shown in Table V. In "moderately hydrated" ticks, $-\Delta^{\circ}\text{C}$ was -0.768 . In "rehydrated" ticks, the measured freezing point was an insignificantly different -0.684 . On the other hand, the freezing point in "severely desiccated" ticks decreased to a significantly different ($p < 0.005$) -1.019 . Of interest, rehydration caused an increase in haemolymph volume (as indicated above) but no significant change in the osmolarity. One may assume that some degree of osmoregulation takes place possibly by the mobilization of osmotically active solutes into the haemolymph. On the other hand, "moderately hydrated" and "severely desiccated" specimens exhibited an osmolarity change without a significant change in blood volume.

TABLE V
 HAEMOLYMPH FREEZING POINT DEPRESSION ($-\Delta^{\circ}\text{C}$) OF "MODERATELY HYDRATED",
 "SEVERELY DESICCATED" AND "REHYDRATED" TICKS

Experimental Condition	N	% wt. Δ after Rehydration or Desiccation	$-\Delta^{\circ}\text{C} \pm \text{S.D.}$	Significance
"Moderately hydrated" ticks				
Mean	12	-1.6%	-0.768 \pm 0.187	
"Rehydrated" ticks				
Mean	9	+14.3%	-0.684 \pm 0.168	*p=0.4 - 0.2
"Severely desiccated" ticks				
Mean	15	-13.0%	-1.019 \pm 0.183	*p < 0.005

**p < 0.001

*Comparison of "moderately hydrated" ticks to "rehydrated" ticks and "moderately hydrated" ticks to "severely desiccated" ticks (t-test)

**Comparison of "rehydrated" ticks to "severely desiccated" ticks (t-test)

Potassium Concentration

The potassium concentration measured in the haemolymph of "moderately hydrated" ticks was 22.0 mM/l, 21.0 mM/l in "rehydrated" ticks and an insignificantly different 29.5 mM/l in "severely desiccated" ticks (Table VI). Therefore, despite large fluctuations in body water and haemolymph volume the concentration of potassium remained relatively constant.

Sodium Concentration

The average concentration of sodium in the haemolymph of "moderately hydrated" ticks was 294.0 mM/l (Table VII). Although the water content and the blood volume increased substantially during rehydration, the observed concentration of sodium was only 274.5 mM/l. There is no significant difference between these two averages. However, the concentration increased significantly ($p < 0.05$) to 408.0 mM/l during "severe desiccation". Because Na is the most abundant inorganic cation present, these facts are of considerable importance to the overall salt and water balance of the tick.

Magnesium Concentration

In "moderately hydrated" ticks the concentration of Mg was 13.7 mM/l (Table VIII). The concentration decreased to a significantly lower ($p < 0.05$) 6.8 mM/l in "rehydrated" ticks which in "severely desiccated" ticks was only 18.5 mM/l, insignificantly different from that of the "moderately hydrated" ticks.

TABLE VI
 HAEMOLYMPH CONCENTRATION OF POTASSIUM OF "MODERATELY HYDRATED",
 "SEVERELY DESICCATED", AND "REHYDRATED" TICKS

Experimental Condition	N	% wt. Δ after Rehydration or Desiccation	K ⁺ concentration (mM/l) \pm S.D.	Significance
"Moderately hydrated" ticks				
Mean	12	-0.1%	22.0 \pm 11.8	
"Rehydrated" ticks				
Mean	17	+14.1%	21.0 \pm 10.7	*p > 0.5
"Severely desiccated" ticks				
Mean	16	-14.2%	29.5 \pm 19.5	**p=0.2-0.1 *p=0.4 - 0.2

*Comparison of "moderately hydrated" ticks to "rehydrated" ticks and "moderately hydrated" ticks to "severely desiccated" ticks (t-test)

**Comparison of "rehydrated" ticks to "severely desiccated" ticks (t-test)

TABLE VII
 HAEMOLYMPH CONCENTRATION OF SODIUM OF "MODERATELY HYDRATED",
 "SEVERELY DESICCATED", AND "REHYDRATED" TICKS

Experimental Condition	N	% wt. Δ after Rehydration or Desiccation	Na ⁺ concentration (mM/l) \pm S.D.	Significance
"Moderately desiccated" ticks				
Mean	24	-2.1%	294.0 \pm 118.1	
"Rehydrated" ticks				
Mean	11	+13.8%	274.5 \pm 44.0	*p > 0.5
"Severely desiccated" ticks				**p < 0.02
Mean	8	-12.5%	408.0 \pm 173.9	*p < 0.05

*Comparison of "moderately hydrated" ticks to "rehydrated" ticks and "moderately hydrated" ticks to "severely desiccated" ticks (t-test)

**Comparison of rehydrated ticks to "severely desiccated" ticks (t-test)

TABLE VIII
 HAEMOLYMPH CONCENTRATION OF MAGNESIUM OF "MODERATELY HYDRATED",
 "SEVERELY DESICCATED", AND "REHYDRATED" TICKS

Experimental Condition	N	% wt. Δ after Rehydration or Desiccation	Mg ⁺⁺ Concentration (mM/l \pm S.D.)	Significance
"Moderately hydrated" ticks				
Mean	9	-2.2%	13.7 \pm 9.0	
"Rehydrated" ticks				
Mean	11	+17.0%	6.8 \pm 2.9	*p < 0.05
"Severely desiccated" ticks				**p < 0.02
Mean	11	-13.0%	18.5 \pm 12.5	*p=0.4-0.2

*Comparison of "moderately hydrated" ticks to "rehydrated" ticks and "moderately hydrated" ticks to "severely desiccated" ticks (t-test)

**Comparison of "rehydrated" ticks to "severely desiccated" ticks (t-test)

Calcium Concentration

The concentration of calcium in the haemolymph was very nearly the same as that of magnesium. In "moderately hydrated" ticks the concentration was 12.7 mM/l and in "rehydrated" ticks 6.7 mM/l (Table IX). The two averages are significantly different at the $p < 0.01$ level. In "severely desiccated" ticks the measured concentration was 14.6 mM/l which was not significantly from that observed in "moderately hydrated" ticks.

TABLE IX
 HAEMOLYMPH CONCENTRATION OF CALCIUM OF "MODERATELY HYDRATED",
 "SEVERELY DESICCATED", AND REHYDRATED TICKS

Experimental Condition	N	% wt. Δ after Rehydration or Desiccation	Ca ⁺⁺ concentration (mM/l) \pm S.D.	Significance
"Moderately hydrated" ticks				
Mean	17	+0.3%	12.7 \pm 7.2	
"Rehydrated" ticks				
Mean	11	+14.1%	6.7 \pm 2.5	*p < 0.01
"Severely desiccated" ticks				
Mean	7	-13.1%	14.6 \pm 8.2	*p > 0.5 **p < 0.005

*Comparison of "moderately hydrated" ticks to "rehydrated" ticks and "moderately hydrated" ticks to "severely desiccated" ticks (t-test)

**Comparison of "rehydrated" ticks to "severely desiccated" ticks (t-test)

CHAPTER V

DISCUSSION

In these experiments, it was desirable to confirm that changes in the weights of the animals were mostly indications of gains or losses of body water. The information derived on changes in total body water content confirm that almost all the observed weight changes were caused by gains or losses of water. Furthermore, the data on changes in haemolymph volume indicate that desiccation and rehydration can have substantial effects on the amount of haemolymph present.

There are different explanations on the role of the lone star tick's regulatory abilities depending on how one defines "normal" ticks. If "moderately hydrated" ticks (whose weight change was between -5 to +5 percent of the tick's post-desiccated weight) are defined as "normal" and compared to "severely desiccated" and "rehydrated" ticks, the haemolymph volume percent does not show a significant change after desiccation but does show a significant change after rehydration. Therefore, during rehydration water must be absorbed into the haemolymph from the atmosphere. During "severe desiccation", the water in the tissue is lost and the water in the blood is almost unchanged and is thus possibly compensated for by movement of water from the gut or other tissue into the blood. Since the haemolymph osmolarity remained almost constant as the haemolymph volume percent increased, it is possible that solutes have been added to the haemolymph. After assaying the concentrations of the

four inorganic cations, Na, K, Mg, Ca, it appears that ticks are unable to regulate Mg and Ca during rehydration, but can regulate Na and K. Because Na is the most abundant cation in the haemolymph, it must play an important role in haemolymph osmolarity regulation. Because the haemolymph volume percent increased from 8.8 in "moderately hydrated" ticks to 22.7 in "rehydrated" ticks during rehydration, one might expect $\Delta^{\circ}\text{C}$ to increase to about -0.290 in the absence of regulation, but this was not the case (-0.684). Since amino acids and chloride are most assuredly important constituents of the haemolymph (Djajakusumak and Miles, 1966), they may have moved into the haemolymph to help regulate the haemolymph osmolarity.

In "severely desiccated" ticks the haemolymph osmolarity increased, the haemolymph volume remained almost unchanged but the total body weight decreased significantly. Sodium was not regulated during desiccation.

Since we noticed that after rehydrating the pre-desiccated ticks, they returned to their original weight and because we maintained the ticks in high humidity prior to the experiment, it may be more appropriate to refer to "rehydrated ticks" as "normal" ticks. If we compare "rehydrated" ticks to "moderately hydrated" and "severely desiccated" ticks on their ability to osmoregulate, the results can be interpreted differently. As water was lost from haemolymph, the "expected" $\Delta^{\circ}\text{C}$ should decrease to about -1.764 in "moderately hydrated" ticks. However, the results show that the lone star tick can regulate its haemolymph osmolarity under "moderately hydrated" conditions, $\Delta^{\circ}\text{C}$ being -0.768 , some 2.3 times higher than the "expected". Additionally, since Na and K concentrations were left almost unchanged, we are left with the

conclusion that ticks have the ability to regulate their haemolymph osmolarity with the help of substantial Na and K movement out of the haemolymph. Because Mg and Ca account for only a small fraction of the total number of solutes, the haemolymph osmolarity was not substantially affected by increasing levels of Mg and Ca. During severe desiccation, Mg, Ca, and Na rose significantly as did the osmolarity but all are less than "expected" in view of the change to the haemolymph volume percent. The $-\Delta^{\circ}\text{C}$ is 1.9 times higher than the "expected".

The combined effects of desiccation and rehydration are depicted in Figure 1. The letter symbols used in the histograms are S to represent "severely desiccated" ticks; M for "moderately hydrated" animals; and H signifies "rehydrated" ticks.

As shown, the water content percent is linearly related to the hydration state of the animal (S, M and H). However, the blood volume percent is little different in S and M ticks even though the entire body water content percent changes significantly. This indicates that when "severely desiccated" ticks become moderately hydrated, almost all the water is taken up by non-haemolymph portions of the body. Conversely, when "moderately hydrated" ticks undergo severe desiccation, much of the net water loss is from parts of the body other than the haemolymph which is another way of expressing the tick's ability to regulate this volume during "severe desiccation". However, as "moderately hydrated" (M) ticks become "fully hydrated", the haemolymph volume increases sharply. Actually, as indicated above, almost all the net water taken up by ticks in this state can be accounted for by an increase in haemolymph volume.

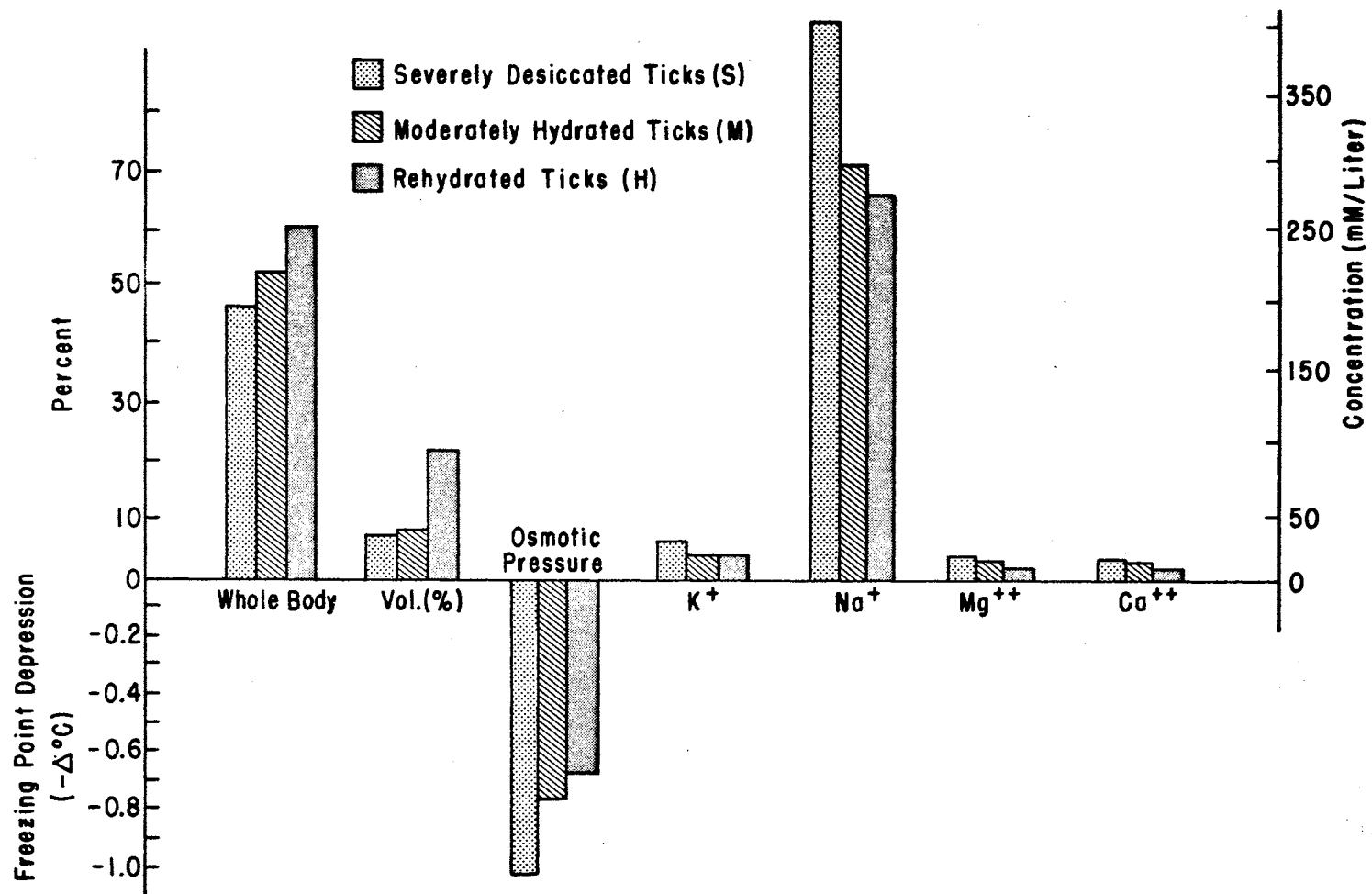


Figure 1. Combined Effects of Desiccation and Rehydration in the Lone Star Tick. The first set of three bars indicates the whole body water content percent. All other sets signify the indicated property of the haemolymph.

If we next turn to the changes in freezing point and Na (the most abundant inorganic cation), we see sharp increases in the $-\Delta^{\circ}\text{C}$ and decrease in Na concentration when severely desiccated ticks become moderately hydrated. This occurs in spite of little change in haemolymph volume percent. Solutes, probably mostly Na, are withdrawn from the haemolymph into other parts of the body. Conversely, when "moderately hydrated" ticks become "severely desiccated", the haemolymph experiences sharp increases in its osmolarity and concentration of Na at the same time the haemolymph volume percent remains relatively constant. This suggests that solutes, probably mostly Na ions, move from other parts of the body to the haemolymph and seem to be an important part of a mechanism aimed at regulating the volume.

On the other hand, when "moderately hydrated" ticks become "fully hydrated", there is little change in the haemolymph osmolarity and haemolymph concentration of Na even though there is a substantial increase in haemolymph volume percent. This suggests that much of the net water that ultimately enters the haemolymph enters concurrently with solutes many of which are probably Na ions.

During all states of hydration, the concentration of potassium remained relatively constant indicating that the lone star tick has an efficient means of regulating this ion. On the other hand, the tick appears unable to regulate Mg and Ca because the concentration of both rose and fell inversely at approximately the same rate as the blood volume percent.

The results clearly show that the level of hydration has profound effects on properties of the haemolymph. The fluctuating concentration of Na, in particular, appears to be linked to concurrent movements of

water between haemolymph and non-haemolymph parts of the body. Also since Na is initially removed from the haemolymph by "severely desiccated" ticks in the process of becoming "moderately hydrated" and the increase in water content during these events is mostly to non-haemolymph parts of the tick, it is tempting to believe that sodium may play as yet some unknown but important role in the ability of this arthropod to absorb water from subsaturated atmospheres.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This study describes the effects of desiccation and rehydration on the water content, haemolymph volume percent, osmolarity and concentrations of Na, K, Mg and Ca in the haemolymph of the lone star tick, Amblyomma americanum (L.).

The water content percentage of "severely desiccated", "moderately" and "fully hydrated" ticks were 46.0, 52.8 and 60.3 percent respectively. The lowest and highest of these were near the minimum and maximum possible.

The haemolymph volume percent of "severely desiccated" ticks was regulated near the level of "moderately hydrated" ticks despite significant decreases in total body water content and increases in blood osmolarity and concentrations of sodium. Conversely, the change from "severely desiccated" to "moderately hydrated" ticks can be viewed as causing an increase in total body water, decrease in blood osmolarity and sodium, but little change in haemolymph volume percent.

Most of the water taken up by "moderately hydrated" ticks (while becoming "fully hydrated") was added to the haemolymph. At the same time, there was little change in the blood osmolarity or haemolymph concentration of sodium.

The concentration of potassium was regulated over the full range of desiccating and hydrating conditions. The lone star tick appeared

unable to regulate its haemolymph concentrations of Ca and Mg; both fluctuated at the same rate, but inversely as the haemolymph volume percent.

It appears that a carefully controlled movement of solutes (Na the predominant cation) between haemolymph and non-haemolymph tissue is intimately linked with haemolymph volume regulation and movement of water into the haemolymph during hydration.

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