SODIUM, POTASSIUM, CHLORIDE AND WATER BALANCE IN THE FEEDING LONE STAR TICK, AMBLYOMMA AMERICANUM (LINNEAUS)

By

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PREFACE

This investigation was designed to study the total water content percent, osmolarities of the haemolymph and saliva, and movement of sodium, potassium, and chloride ions from the host blood to the tick as a whole, gut lumen, haemolymph and saliva of the feeding female lone star tick, Amblyomma americanum (L.) and to determine if salivary secretion functions in salt and water balance regulation.

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CHAPTER I

INTRODUCTION

Maintenance of salt and water balance in the tick is of interest because of its unusual life cycle, which requires it to conserve and/or obtain water between bloodmeals and the equally important but opposite problem of eliminating large amounts of excess fluid during feeding.

The homeostatic condition of salt and water balance is maintained by regulation of the internal bathing medium. The cells and tissues function most efficiently within a narrow range of osmotic concentrations. The osmotic regulatory function of arthropods has been extensively investigated and has been the subject of numerous reviews (Berridge, 1968a; Stobbart and Shaw, 1964; Barton-Browne, 1964; Buck, 1953; Craig, 1960; Edney, 1957). These studies have indicated that the Malpighian tubules and rectum are important in osmoregulation of insects. In addition, the cuticle (Beament, 1964, 1965; Locke, 1965; Lees, 1948) and the respiratory system (Bursell, 1957) also play a part in retaining body water. Likewise, the coxal glands in argasid ticks (Araman and Said, 1972; Lees, 1946b) and salivary glands in ixodid ticks (Tatchell, 1967, 1969; Kaufman and Phillips, 1973a) eliminate excess water during feeding.

The ultimate aim in this study was to investigate the total body water content percent of the adult female feeding lone star tick, Amblyomma americanum (L.) and to trace the movements of sodium,

potassium and chloride ions from the host blood to this feeding tick as a whole, gut lumen, haemolymph and determine more precisely the role of salivary secretion in maintaining salt and water balance in this species of feeding ixodid tick.

CHAPTER II

REVIEW OF THE LITERATURE

The lone star tick, Amblyomma americanum (L.), is one of the most important tick species in the Ozark region, as well as the entire United States. In the Ozark region dense brushy-type vegetation offers an excellent habitat for this pest. The abundance of this pest has resulted in slow economic development of the affected areas, reduced tourist business, and wildlife deaths and incapacitation of man due to tick-borne diseases.

The lone star tick must depend on three separate bloodmeals in order to complete its life cycle. Under field conditions these bloodmeals are probably derived from three different hosts (Diamant and Strickland, 1965). Very few warm-blooded animals and birds are exempt from attack by this pest (Herms and James, 1961). During the blood-feeding process, the mouthparts of this pest are deeply embedded in the flesh of man and other animals, which sometimes causes severe inflammation. Several diseases are transmitted by this tick including Rocky Mountain spotted fever, tularemia, Q fever, lone star virus, Bullis fever, and tick paralysis.

An important function of ticks and other bloodsucking arthropods is water balance (Lees, 1946a). Some investigations have shown that many species of arthropods resist desiccation by absorbing water from subsaturated atmospheres (Hafez et al., 1970a,b; Edney, 1966; Wharton and

Kanungo, 1962; Noble-Nesbitt, 1969; Knulle, 1966; Lees, 1946a; Sauer and Hair, 1971). It is also known that certain species of arthropods convert dry weight reserves to metabolic water to replace water lost via transpiration during desiccation (Edney, 1966; Noble-Nesbitt, 1969).

The data of Lees (1946a) showed that the sheep tick, Ixodes ricinus (L.), lost 50 percent of its original weight within 24 hours when kept under zero percent RH. Significantly, ticks survived for 2 to 3 months at 90 percent RH because water from the cuticle was not given up to the highly humid atmosphere. Feldman-Muhsam (1947) showed that Hyalomma savignyi Gerv. ticks survived only a few days at 20 percent RH and 17.5°C, but were alive after approximately seven months when held in an atmosphere of 95 percent RH.

The osmoregulatory function of arthropods is paramount in maintaining the constancy of the internal environment or haemolymph. The haemolymph bathes the cells of most tissues and circulates throughout the body serving as a reservoir of water and soluble components. This involves not only the control of the total osmotic pressure but also of the concentration of the individual inorganic ions and the many organic solutes present.

Under different environmental conditions such as desiccation, hydration, feeding, and starvation, arthropods are known to tolerate or regulate their haemolymph volume and constituents by moving salts and water and changing the relative amounts of soluble proteins and amino acids in the haemolymph (Djajakusumah and Miles, 1966; Wall, 1970; Pichon, 1970; Shih et al., 1973; 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 and selective

reabsorption from the rectum of the hindgut. Movement of water and salts may occur across the body surface, through the gut, or through the excretory system. The movements may be purely passive and down osmotic gradients or through active transport mechanisms in order to maintain the insect in a steady state with respect to its water and salt content.

The excretory system of insects performs a dual function, since it is concerned not only with the elimination of metabolic waste products, but with the maintenance of salt and water balance as well. In the great majority of insects the excretory system comprises a number of Malpighian tubules, generally lying free in the haemocoel, which open into the alimentary canal at the junction of the midgut and hindgut. They are closed at the distal end and are made of a single layer of epithelial cells which usually have a conspicuous striated border on the luminal side.

Many Malpighian tubules appear to be similar in structure throughout their entire length. But the tubules of some species show regional differentiation in tubular structure. The tubules of Rhodnius prolixus Stal. (Wigglesworth, 1931), where there is an abrupt transition between the distal and proximal portions, are characterized by a change in the striated border from the honeycomb to the brush-border type.

Regional differentiation of function in the tubules has been found in certain insects. According to Wigglesworth (1931), uric acid precipitates in the proximal portion of the tubules of R. prolixus. Analysis of the luminal contents in the two regions shows differences in ionic composition (Ramsay, 1952). The distal region has the usual pattern of a high potassium concentration and a sodium concentration below that of the haemolymph. The differences in concentration established by the

activity of the distal portion are degraded in the proximal region. Thus, the osmotic pressure falls.

Ramsay (1953b, 1954, 1955b, 1956) proposed that the active transport of potassium is fundamental to the production of the tubule fluid, and is the prime mover in generating the flow of urine. Shaw and Stobbart (1963) suggested that water might be carried by frictional interaction with the actively transported potassium ions, just as Diamond (1962) has shown that water is moved against an osmotic gradient by the active transport of sodium chloride in the gall bladder.

The rectum plays a major role in the modification of the urine fluid secreted by the Malpighian tubules. In the freshwater insects the rectal fluid is hypoosmotic to the haemolymph, and the major inorganic ions of the haemolymph are present in low concentration (Ramsay, 1950, 1953a; Shaw and Stobbart, 1963; Sutcliffe, 1961). The saltwater species produce a rectal fluid which is strongly hyperosmotic to the haemolymph (Ramsay, 1950; Sutcliffe, 1960). Likewise, the rectal fluid of the terrestrial insects may be highly concentrated for water conservation.

Rectal reabsorption of water is apparent in Phasmidae (Ramsay, 1955a). The high value of the rectal fluid osmotic pressure show that water is reabsorbed against a large osmotic gradient. The mechanism of water reabsorption in the rectum of locust and blowfly was extensively investigated by Phillips (1961, 1964a, b, c). Phillips (1964a, b, c) reported that in starved locusts supplied with tap water more salt than water was reabsorbed; while supplied with hypertonic saline, water was reabsorbed in the rectum. The rate of rectal water reabsorption may be controlled by an antidiuretic hormone (Mills, 1967; Wall, 1967).

In the larva of Tenebrio molitor L. the permeability of the rectal

wall to sodium was demonstrated by Patton and Craig (1939), who showed that labelled sodium in the Malpighian tubules fluid passed back through the rectal wall from the rectum. Ramsay (1953a) found that both sodium and potassium are reabsorbed in the rectum of mosquito larvae.

Bloodsucking arthropods eliminate much of the water content of the ingested blood together with a proportion of the dissolved electrolytes which would otherwise cause osmotic and ionic imbalance. The regulatory mechanisms for the maintenance of the ionic composition of the internal bathing medium depend on the control of the normal water content; the processes of ionic regulation and water balance are interrelated.

In R. prolixus (Maddrell, 1963) up to 150 µl of hypotonic urine can be produced within four hours of feeding. Similarly, large amounts of the bloodmeal are eliminated by mosquitoes (Boorman, 1960) and tsetse flies (Lester and Lloyd, 1928).

Tick feeding has been extensively investigated and has been the subject of several reviews (Sutton and Arthur, 1962; Arthur, 1965, 1970). It is generally believed that the ingested meal is concentrated by the elimination of excess water during feeding.

The coxal organs of argasid ticks can secrete over 120 µl of hypotonic fluid in less than an hour and exercise a measure of control over the haemolymph chloride concentration (Araman and Said, 1972).

Ixodid ticks lack coxal glands and it was generally assumed that they concentrated the bloodmeal primarily by evaporative water loss through the cuticle (Lees, 1946a). Gregson (1957) first reported that feeding ixodid ticks secrete a clear fluid into the host and suggested that this may represent elimination of excess water from the ingested

bloodmeal. The rapid engorgement phase in ixodid ticks which occurs during the last 12 to 24 hours of attachment is characterized by rapid ingestion of a large volume of blood and visible expansion of the cuticle (Lees, 1947, 1952).

Tatchell (1967) injected tritiated water into the haemocoel of feeding adult cattle ticks, Boophilus microplus (Canestrini), and later retrieved it from the host. He concluded that during engorgement large amounts of water which have passed across the gut epithelium into the haemolymph are returned to the host. Later, Tatchell (1969) indicated that the salivary secretory mechanism is able to exercise some regulatory control over the haemolymph osmotic pressure and the ionic concentration in the engorged cattle tick. During feeding of the adult tick Dermacentor andersoni Stiles, about 80 percent of the total bloodmeal is excreted. Salivation accounts for approximately 75 percent of the total water excreted from the large bloodmeal (Kaufman and Phillips, 1973a).

According to Arthur (1970) the course of feeding in I. ricinus is made up of three phases, the last of which consists primarily of the ingestion of blood. Snow (1970) found that all instars of H. anatolicum Koch concentrate the ingested blood by eliminating excess water. Other workers (Seifert et al., 1968; Lees, 1946a; Balashov, 1972a) have demonstrated that various ticks concentrate the bloodmeal by eliminating excess water.

Kaufman and Phillips (1973b) postulated that, in vitro, the salivation of D. andersoni occurs by means of a secretory rather than a filtration-resorption mechanism. They also suggested that salivation in this tick involves passive movement of water coupled to the active transport of solutes and that a specific chloride pump may be principal driv-

ing force of fluid secretion (Kaufman and Phillips, 1973c).

Oral secretion of ticks can have several effects on the host according to a recent report by Kirkland (1971), a few of which have been reported by Lavoipierre and Riek (1955) and Hoogstraal (1966, 1967). By knowing the effects of feeding on the concentration of haemolymph electrolytes it may be possible to relate the concentration of an electrolyte in the haemolymph to the activity level of the organism. Hoyle (1954) and Brady (1968) indicated that the activity level of insects may be controlled by the concentration of potassium in its blood.

CHAPTER III

MATERIALS AND METHODS

Experimental Animals

Animals used in all experiments were partially fed and fully engorged (replete) female lone star ticks, Amblyomma americanum (L.), which were reared in the Oklahoma State University Medical Entomology Laboratory. Stanchioned sheep served as hosts for the adult ticks. The unfed adult ticks were confined in cells which consisted of a piece of orthopedic stocking that was attached to the host by formica contact cement. The areas within the cells were sheared to facilitate rapid attachment to the animal. Equal numbers (40) of males and females were allowed to commence feeding and at daily intervals a few ticks which either were still feeding or had fully engorged and voluntarily detached from the sheep were collected. All experiments were conducted within one hour after removing the ticks from the host. Prior to experiments, ticks were weighed on an analytical balance sensitive to the nearest 0.01 mg.

Collection of Haemolymph

Haemolymph of the experimental ticks was collected from wounds (made by cutting off legs) under a stereomicroscope with finely drawn precalibrated glass capillaries (0.2 µl for sodium and potassium, 1.0 µl for chloride analysis). The samples were discarded if they became

contaminated with gut contents. Samples were transferred into pre-measured volumes of deionized water for sodium and potassium analysis with a Beckman 440 atomic absorption spectrophotometer. The chloride analysis was made directly without dilution with deionized water and measured by the Coulombic silver chloride precipitation method using a Fiske/Marius microchlor-o-counter (Burton et al., 1972). Because of the limited amount of haemolymph in small feeding ticks, several ticks of approximately the same weight were oftentimes required for one chloride analysis.

Procedure for Sampling Saliva

Attempts which were first made by injection of 10^{-5} M or 10^{-3} M of adrenaline saline (Sauer et al., 1974) failed to stimulate secretion. Finally, a copious secretion was elicited when concentrations of 10^{-2}M adrenaline were injected into the tick. Ticks were placed ventral side up; and by carefully pushing the fine needle of a fifty µl syringe beneath the integument and toward the anterial part of the tick, the solution was inserted into the tick. Salivary secretions were collected within five minutes after injection of adrenaline with finely drawn, calibrated capillaries (1.0 µl) placed over the chelicerae and hypostome of the partially fed or fully engorged female ticks. Samples of saliva were appropriately diluted with deionized water for sodium and potassium analysis. Chloride determination was made without dilution as described previously.

Sampling of Gut Fluid

The contents of the experimental ticks were teased out into a small

glass dish by making a lateral incision of the tick integument with a single edge razor blade. A portion of the gut of the ticks was carefully collected into a preweighed vial with a probe. The weight of the gut fluid was determined by substracting the weight of the vial from that of the vial and its contents. Analysis of the gut fluid was carried out following complete solution of the gut fluid in an appropriate amount of concentrated nitric acid and subsequent dilution with deionized water for sodium and potassium assays. Measurement of chloride concentration was made without further dilution with deionized water.

Analysis of Whole Tick

Whole ticks were homogenized in an appropriate amount of concentrated nitric acid and subsequently diluted with deionized water before assaying for sodium and potassium. No further dilution with deionized water was required for chloride determination after the ticks were homogenized in concentrated sodium hydroxide.

Freezing Point Depression

Osmolarity determinations of collected haemolymph and saliva of experimental ticks were measured with a Clifton Technical Physics Nanoliter Osmometer, expressed as the freezing point depression ($-\Delta°C$) sensitive to the nearest $\pm$ 0.001°C (Frick and Sauer, 1973). Because of the minute quantity of fluid required, sufficient fluid could be obtained even from the smallest ticks.

Water Content

After determining the weight, individual ticks in glass vials were placed in a drying oven at 105°C and weighed again every 12 hours until a constant weight was obtained. The difference between wet and dry weights was taken as the water content of the ticks.

Sheep Blood

The neck area of the sheep was sheared to facilitate drawing blood out of the vein with a syringe. The sheep blood was then transferred into an evacuated glass container through a rubber stopper which contained 0.04 ml 15 percent soluble EDTA (Ethylene Diamine Tetra-acetic Acid) as an anticoagulant and potassium sorbate as an antimycotic agent. The blood was slowly, constantly agitated for five minutes to prevent coagulation. Samples of sheep blood were diluted with deionized water for sodium and potassium analysis, whereas chloride was assayed without dilution.

CHAPTER IV

RESULTS

Water Content Percent

The total body water content percent of adult female unfed lone star ticks was reported to be 52.8 (Shih et al., 1973). This contrasts sharply with the 69.0 seen in the tick at the beginning of the blood-meal (Fig. 1). With the onset of rapid engorgement, the water content percent decreased to 63.3 and decreased further to 57.5 prior to and just after completion of engorgement.

If the whole feeding process is divided into 3 phases (i.e., initiation of bloodsucking, beginning of rapid engorgement and final engorgement), the water content percent decreased proportionally with increasing weight of the ticks and maintained a rather narrow constant range within each phase.

Sodium Concentration

Analysis of ion concentrations in the host (sheep) is shown in Table I. The sodium concentration of the sheep whole venous blood was 110.0 mequiv./l. The average concentration of sodium in the whole tick was maintained at 62 mequiv./l (Fig. 2) during the entire feeding process and just after detachment from the host. Likewise, sodium concentration in the gut fluid was about 42 mequiv./l throughout the feeding process. During the initial stage of feeding the sodium concentration in the

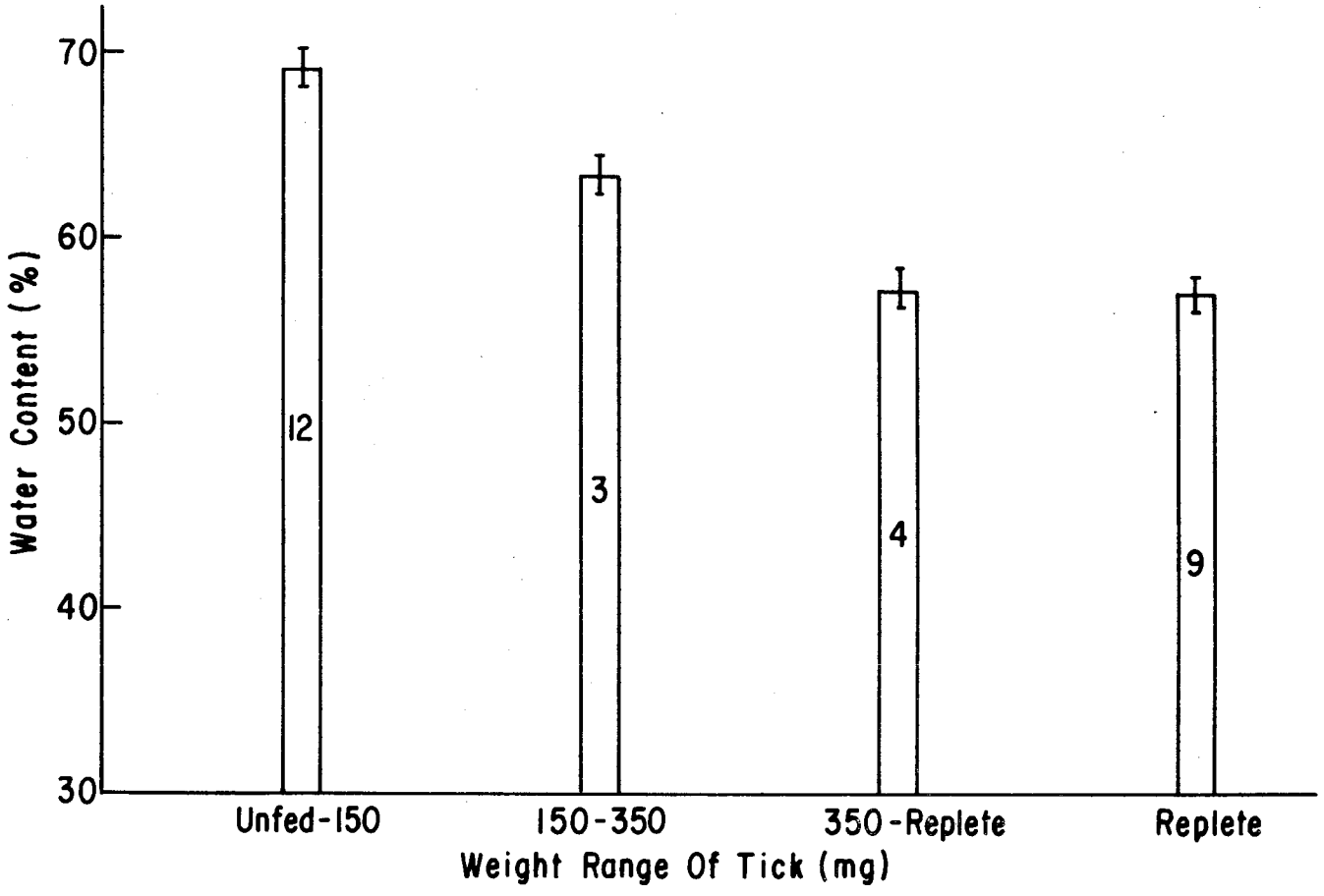

Figure 1. The Whole Body Water Content Percent of the Adult Female Lone Star Tick during Different Phases of Feeding. Vertical lines represent the ± S. D. of the means. Numbers in bars indicate the no. of assays.

TABLE I

THE IONIC CONCENTRATIONS OF THE WHOLE BLOOD, SERUM OR PLASMA AND ERYTHROCYTES IN SHEEP BLOOD

	Concentration (mequiv./l)		
	Na^+	K^+	Cl^-
Whole blood*	110.0	31.2	95.1
Serum or plasma**	151-160	4.8-5.9	116
Erythrocytes**	98	46	78

* Average of 5 readings of blood obtained from 2 sheep.

** From Altman (1961).

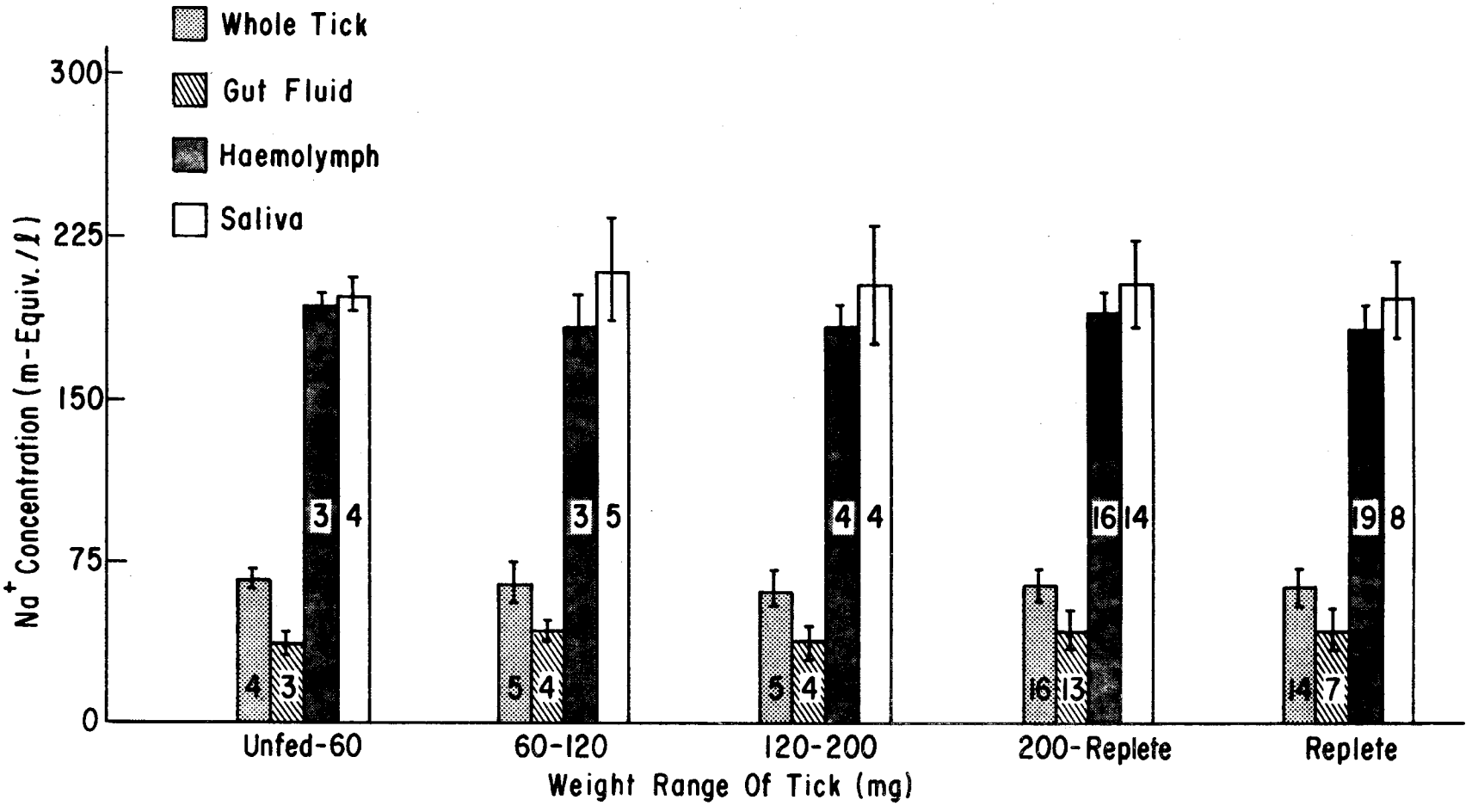

Figure 2. Sodium Concentration in the Whole Tick and Body Fluids of the Adult Female Lone Star Tick with Time during the Feeding Process. Vertical lines represent ± S. D. of the means. Numbers in bars indicate the no. of assays.

haemolymph of the tick fell from 294 mequiv./l in the unfed tick (Shih et al., 1973) to about 194 mequiv./l. It then decreased slightly to about 184 mequiv./l and remained at this level until repletion. The sodium concentration measured in the saliva was about 200 mequiv./l over the entire course of salivation. Interestingly, the sodium concentrations were much higher in the haemolymph and saliva than in the whole tick and gut fluid.

Potassium Concentration

The potassium concentration measured in the whole blood of the host was 31.2 mequiv./l. The average potassium concentration in the whole tick was at a higher value of 83 mequiv./l during initial feeding periods (Fig. 3), but decreased to about 46 mequiv./l at the onset of rapid engorgement. This level was maintained until repletion. Similarly, the gut fluid had a fairly high concentration of potassium at about 126 mequiv./l initially. It then fell sharply and was maintained at 51 mequiv./l throughout the rest of the engorging process. The concentration of potassium in the haemolymph of the unfed tick was reported to be 22 mequiv./l (Shih et al., 1973). It decreased and remained rather constant at about 15 mequiv./l throught the feeding period. Comparisons of the concentrations of potassium in the haemolymph and saliva indicated no significant difference. In contrast to the distribution of sodium, the potassium concentrations were lower in the haemolymph and saliva than in the whole tick and gut fluid.

Chloride Concentration

The chloride concentration in the whole blood of the sheep was 95.1

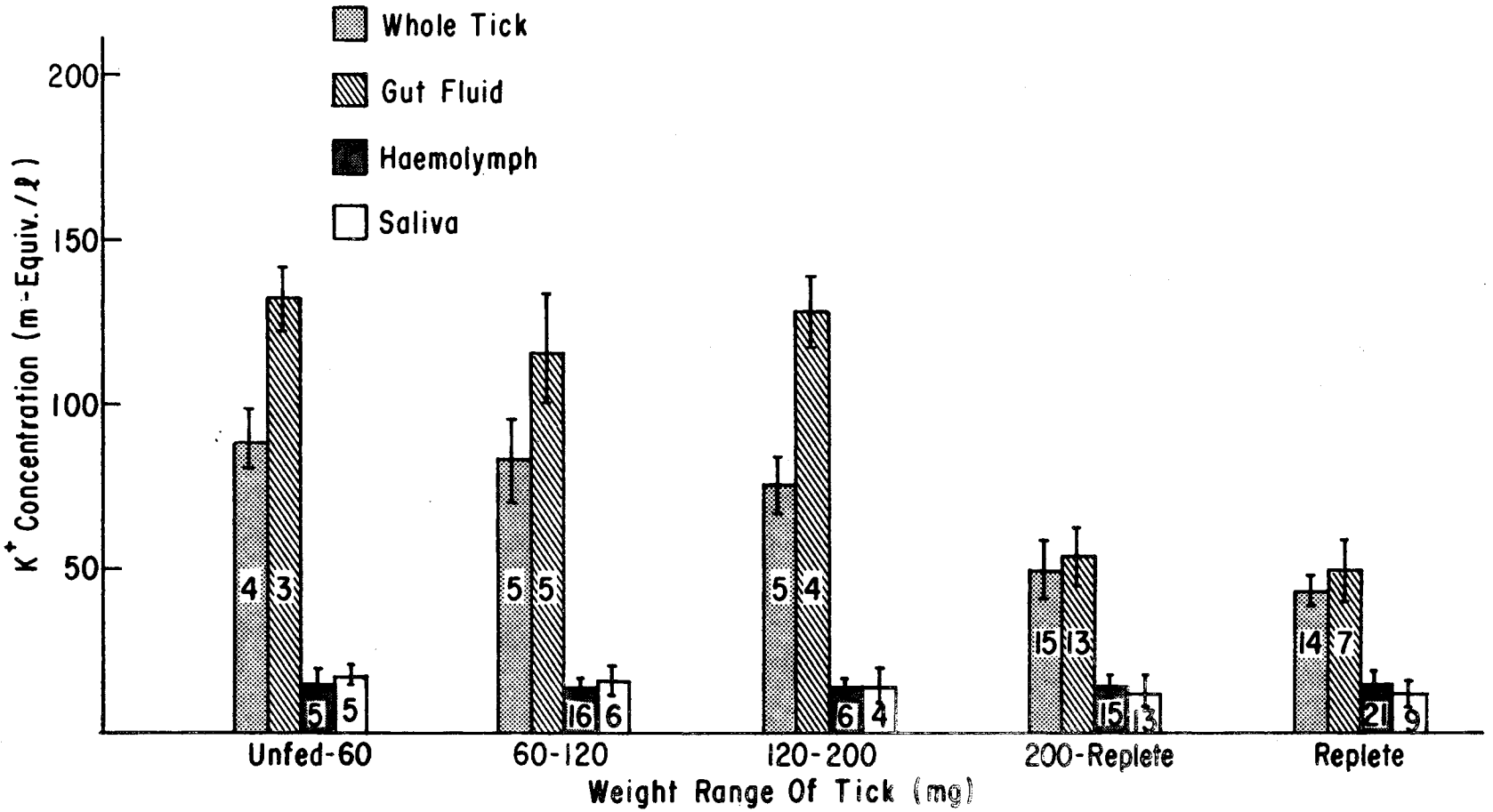

Figure 3. Potassium Concentration in the Whole Tick and Body Fluids of the Adult Female Lone Star Tick with Time during the Feeding Process. Vertical lines represent ± S. D. of the means. Numbers in bars indicate the no. of assays.

mequiv./l. The figures for chloride in the whole tick were not complete; only replete ticks were analyzed with an average of 66 mequiv./l being measured (Fig. 4). The chloride concentration in the gut fluid was maintained at a rather constant value of about 68 mequiv./l during the entire feeding period. In the early stages of feeding, chloride concentrations in the haemolymph were about 150 mequiv./l, but fell to 135 mequiv./l for the remainder of the feeding process. Similarly, the measured chloride concentration of 174 mequiv./l in the saliva at the very beginning of the bloodmeal decreased to 150 mequiv./l for the remainder of salivation. It is worth noting that the pattern of the distribution of chloride is very similar to that of sodium in different compartments of the feeding lone star tick.

Osmotic Pressure

The results of the osmolarity readings in the haemolymph and saliva (measured as freezing point depression, $-\Delta°C$) are shown in Fig. 5. The haemolymph freezing point fell from -0.768°C in the unfed lone star tick (Shih et al., 1973) to -0.693°C initially, and remained at -0.67°C throughout the rest of the bloodfeeding period. Likewise, the measured freezing point in the saliva was about -0.68°C during the entire blood-sucking process. There was no significant difference of osmotic pressure between the haemolymph and saliva.

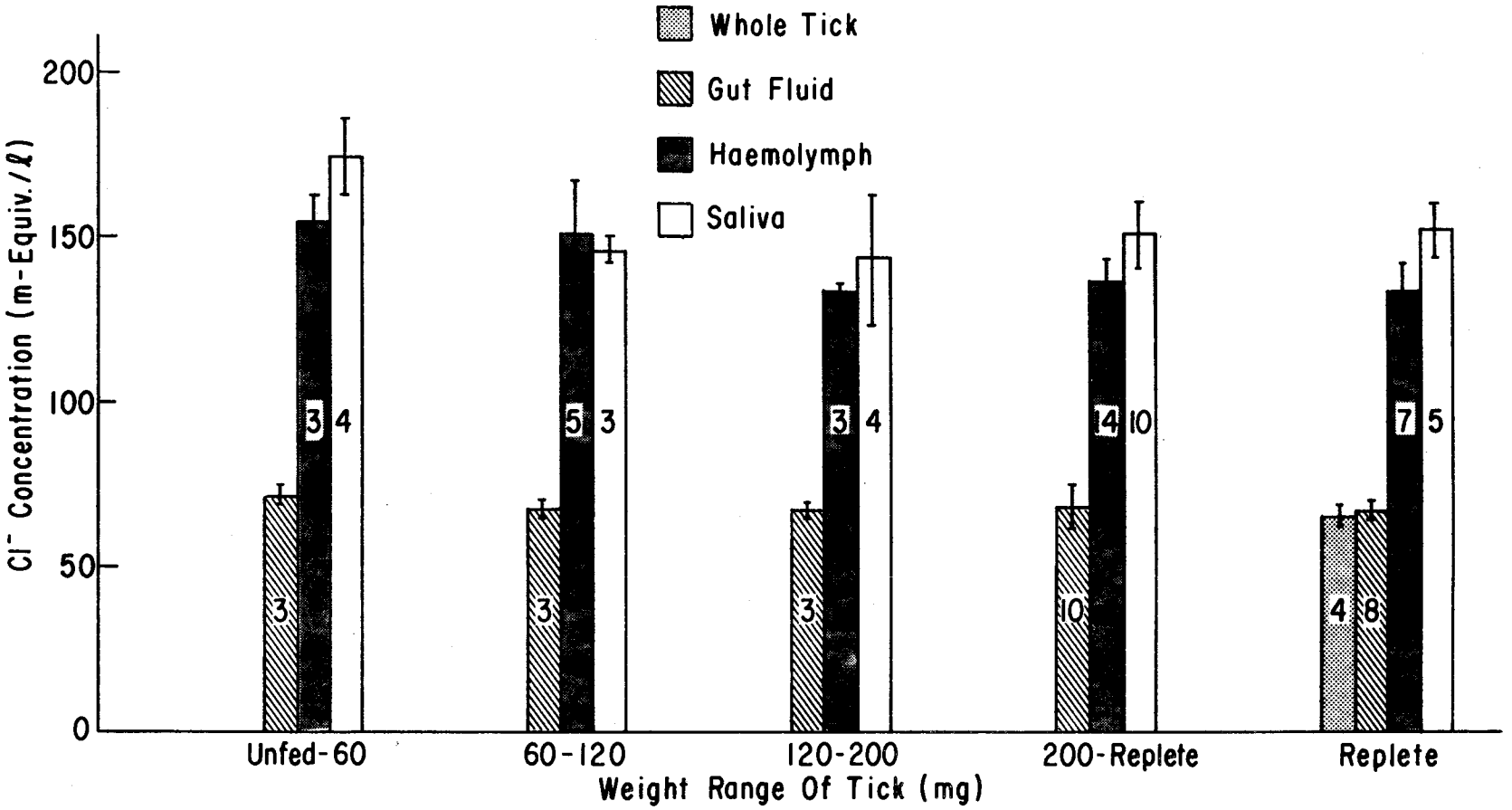

Figure 4. Chloride Concentration in the Whole Tick and Body Fluids of the Adult Female Lone Star Tick with Time during the Feeding Process. Vertical lines represent ± S. D. of the means. Numbers in bars indicate the no. of assays.

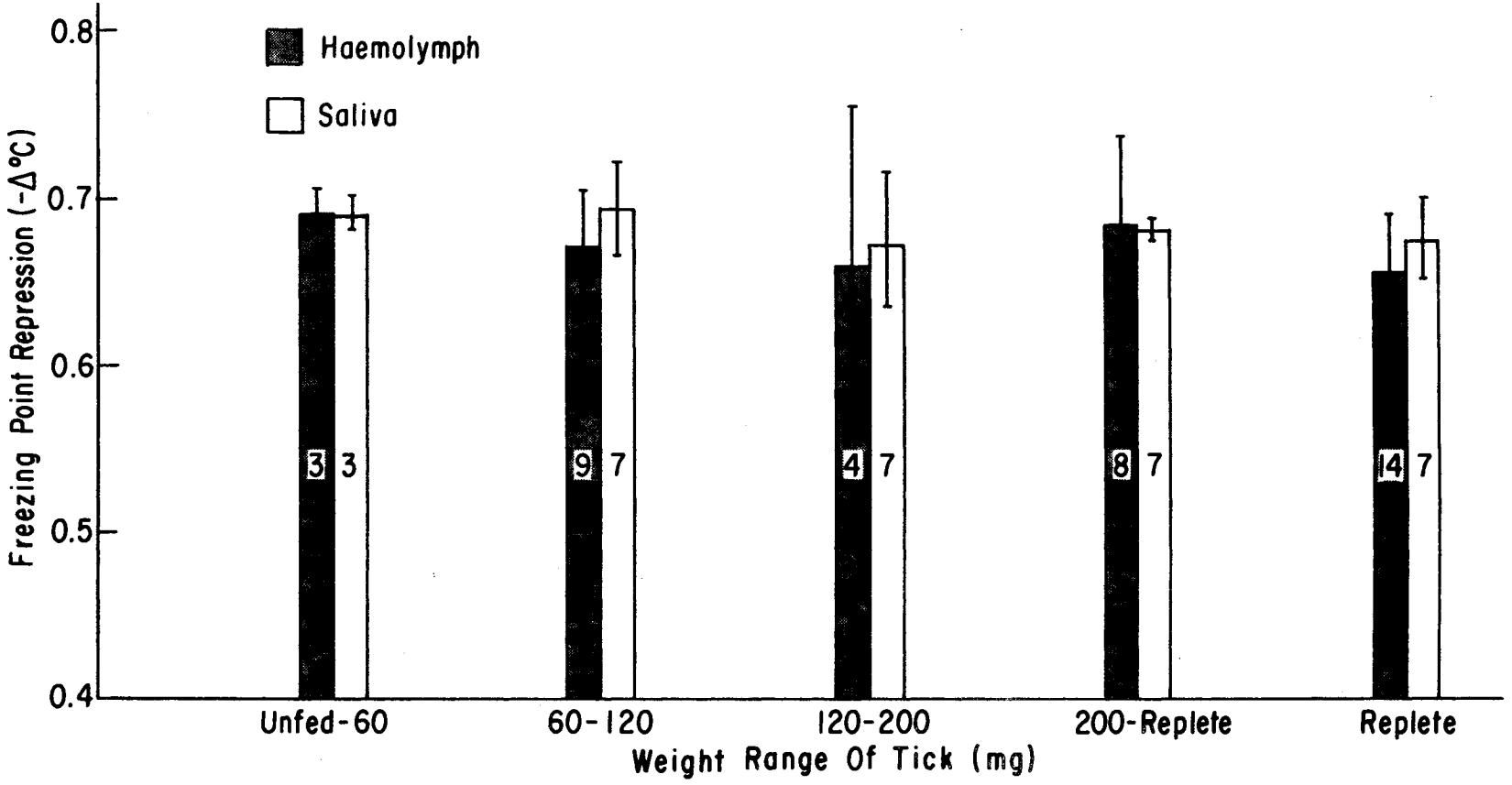

Figure 5. The Osmolarities of the Haemolymph and Saliva with Time in the Adult Female Lone Star Tick during the Course of Feeding. The osmotic pressure is expressed as the freezing point depression (-Δ°C) sensitive to ± 0.001°C. Vertical lines represent ± S. D. of the means. Numbers in bars indicate the no. of assays.

CHAPTER V

DISCUSSION

A study by Belozerov (1967) on the dynamics of whole body water changes during feeding in two species of ixodid ticks, I. ricinus and D. marginatus Sulzer showed that in the initial feeding stage the water content percent of the two species underwent great increases and decreased just prior to and after complete engorgement. Results of the present investigation and Belozerov's research (Table II), coupled with similar experiments on other species of ixodid ticks (Kitaoka, 1961), suggest similar abilities of water balance regulation.

Lees (1946a, 1947) suggested that ixodid ticks eliminate excess water by evaporation through the integument during feeding. However, in the female H. asiaticum Schulze and Schlottke a new epicuticular wax layer is formed by dermal gland secretion during feeding, which appears to restrict water loss from the feeding tick (Balashov, 1972d). Belozerov (1967) indicated that in I. ricinus (sheep tick) the integument permeability increases at the beginning of the feeding process but then decreases and becomes minimal prior to detachment of engorged females. The water content percent decreases as the integument permeability decreases, which suggests that a mechanism other than or in addition to evaporation regulates water balance when the tick ingests the greater part of bloodmeal, because evaporation appears to be insufficient to account for elimination of the excess water.

TABLE II

THE WHOLE BODY WATER CONTENT PERCENT IN 3 SPECIES OF ADULT FEMALE UNFED AND FEEDING IXODID TICKS DURING DIFFERENT STAGES OF FEEDING

Species	Whole Body Water Content (%)			
	Unfed	Initiation of Bloodsucking	Beginning of Rapid Engorgement	Final Engorgement
Amblyomma americanum	52.8*	69.0	63.3	57.5
Ixodes** ricinus	53.2	67.6	67.6	59.0
Dermacentor** marginatus	55.7	65.2	65.2	62.2

* From Shih et al. (1973).

** From Belozerov (1967).

Gregson (1957) first observed that ticks excrete a large amount of saliva into the host during feeding. Later, research was done on the salivary glands of feeding ixodid ticks by Balashov (1972b). He demonstrated that the salivary glands reach maximum sizes in ticks that are ready to ingest the final portion of bloodmeal. Furthermore, it has been confirmed with studies by Tatchell (1967, 1969) and a series of recent reports by Kaufman and Phillips (1973a,b,c) that the salivary glands of ixodid ticks play the principal role in eliminating excess water absorbed with the bloodmeal, and thus, participate in regulating the whole body water content percent, especially during the last phases of the bloodsucking process.

As shown by the results, the lone star tick regulates its body electrolyte composition because the concentrations of sodium, potassium, and chloride in each compartment are maintained at constant levels throughout the feeding process.

The results of the present study support a recent investigation by Marshall (1973) in which the replete adult female Gulf Coast tick, A. maculatum (Koch) demonstrated a net positive transgutepithelial potential, with the serosal (haemolymph) side positive to the luminal side. Because the potential was inhibited when ouabain and other inhibitors were placed on the serosal side of the gut of A. maculatum, Marshall suggested the possibility of a sodium-potassium exchange pump for moving sodium from the gut lumen to the haemolymph of the tick against the electrical gradient. It is reasonable to suspect a similar type of movement across the gut of the feeding lone star tick, A. americanum, because of the measured high concentration of sodium in the haemolymph with respect to the gut fluid. With respect to potassium,

the net movement appears to be from the haemolymph to the gut lumen, since the gut fluid has a higher potassium concentration than the haemolymph.

In the study by Marshall referred to earlier the potential difference decreased when ouabain was placed on the serosal side of the gut epithelium. Since ouabain is believed to be an inhibitor of membrane "pump ATPase", which is associated with a transport mechanism (e.g., Na-K exchange pump) in several vertebrate and invertebrate tissues (Coplon and Maffly, 1972; Berridge, 1968a; Glynn, 1964; Treherne, 1966), the possibility that the potassium ions diffusing into the haemolymph initially down its concentration gradient are again pumped back cannot be ruled out. Balashov (1972c) and Till (1961) reported that the Malpighian tubules of the feeding ticks are not active until after detachment from the host. Therefore, it does not appear too likely that the Malpighian tubules function in potassium excretion in the feeding tick, as is true in several insects studied to date (Ramsay, 1953b; Berridge, 1968b; Maddrell, 1969). However, any definite conclusion cannot be reached without further investigation of potassium movements across the Malpighian tubules of the feeding tick.

The higher chloride concentration in the haemolymph than in the gut fluid could be maintained by active transport against the concentration gradient or by a Donnan equilibrium as suggested by Tatchell (1967). On the other hand, a possible potential difference with the serosal side positive could facilitate the uptake of chloride down its electrical gradient. The net coupling movements of sodium and chloride ions may be the driving force for the movement of water into the haemolymph. It should be pointed out that the concentrations of sodium and chloride

are higher in the host blood than in the gut fluid, but lower than in the haemolymph of the feeding tick; the reverse holds true for potassium. These facts also support our assumption of net movements of sodium and chloride across the gut epithelium into the haemolymph in excess of potassium. The same ionic pattern in the gut fluid and haemolymph has been reported by Araman (1972) in two other feeding ixodid ticks, H. dromedarii Koch and H. anatolicum excavatum Koch (Table III). Based on the preceding statements, a summary of the probable movements of solutes and water across the gut epithelium of the feeding lone star tick is shown in Fig. 6.

It is of interest that both the whole tick and gut lumen have much higher potassium concentrations during the early feeding stages. This suggests that there may be a large proportion of blood cells or non-blood tissues with high levels of potassium being ingested with subsequent rapid hemolysis during the early feeding stages. This was also suggested in an earlier investigation by Tatchell (1969) for the cattle tick B. microplus. It is also possible that in the unfed tick the potassium concentration retained in the gut lumen is fairly high and decreases only gradually with the onset of feeding and then remains rather constant throughout the remainder of the feeding process.

Unlike the great difference between the gut fluid and haemolymph, the ionic concentrations in the haemolymph and saliva showed only slight differences in the feeding lone star tick. As shown in Table III, by comparing the ionic composition of the haemolymph and saliva of A. americanum, B. microplus (Tatchell, 1969), and D. andersoni (Kaufman and Phillips, 1973a), we find that the saliva/haemolymph ratio for chloride in these three species is 1.1 and this is also the case for sodium in

TABLE III

THE MEAN ION CONCENTRATIONS OF THE WHOLE TICK AND BODY FLUIDS, TOGETHER WITH THE OSMOTIC PRESSURES OF HAEMOLYMPH AND SALIVA IN DIFFERENT SPECIES OF ADULT FEMALE FEEDING IXODID TICKS

	Osmotic pressure*	Concentration (mequiv./l) Na^+	K^+	Cl^-
		Amblyomma americanum		
Whole tick		62	46	66
Gut fluid		42	51	68
Haemolymph	-0.67	184	15	135
Saliva	-0.68	200	15	150
		*Boophilus microplus***		
Whole tick		49	37	54
Haemolymph	188	136	15	118
Saliva	231	188	11	128
		*Dermacentor andersoni****		
Haemolymph	375	160	7.5	125
Saliva	356	160	7.5	137
		*Hyalomma dromedarii*****		
Gut fluid		56	57	33
Haemolymph	208.5	186	13	104
		*Hyalomma anatolicum excavatum*****		
Gut fluid		47	56	34
Haemolymph	205.1	186	8	79

* The osmotic pressures of *A. americanum*, *D. andersoni*, and the other 3 species were expressed as freezing point depression, mOsm/l and mM/l NaCl, respectively.

** From Tatchell (1969).

*** From Kaufman and Phillips (1973).

**** From Araman (1972).

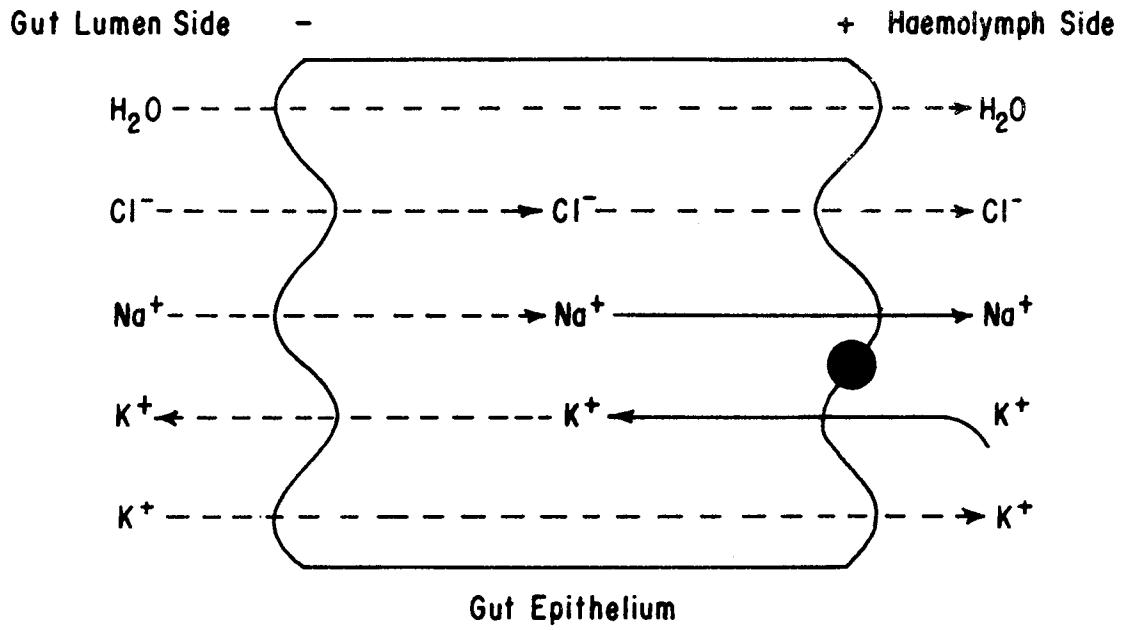

Figure 6. A Postulated Scheme for Movements of Solutes and Water across the Gut Epithelium of the Adult Female Feeding Lone Star Tick. The dark circle represents a possible ouabain sensitive "Na-K" exchange pump. Solid arrows indicate possible active transport of solutes. Broken arrows indicate possible passive movements of solutes or water.

A. americanum. In D. andersoni the saliva/haemolymph ratio for sodium and potassium is insignificantly different from one, and this is also true for potassium in A. americanum. In B. microplus the saliva/haemolymph ratio for sodium is greater than one, and that for potassium is less than one.

The osmotic pressure ratio (saliva/haemolymph) in these three species is 1.01, 1.23, and 0.94, respectively. Kaufman and Phillips (1973c) further demonstrated, in vitro, that D. andersoni produces a hypoosmotic saliva. They postulated that in both in vivo and in vitro the primary secretion is iso- or hyperosmotic, but solute reabsorption occurs somewhere in the main salivary duct. In contrast, the main salivary duct of B. microplus may serve only as a delivery system or may secrete solutes in forming its hyperosmotic saliva. In the present study of the lone star tick we postulate that water movement is a consequence of active transport of ions (possibly coupling of sodium and chloride ions) in the secretory alveoli to form isoosmotic fluid. With this in mind it would be interesting to compare the ultrastructure of the salivary ducts in these three species of ixodid ticks.

It should be emphasized that movement of fluid across certain epithelia has recently been explained by the standing osmotic gradient hypothesis developed by Diamond and Bossert (1967). In a more recent report, Meredith and Kaufman (1973) suggested that the water cell of the group III acinus was a possible candidate for secreting the bulk of fluid in D. andersoni. These authors showed schematically how the standing osmotic gradient was created by the characteristic basal and apical infoldings of the acinus to form the nearly isoosmotic or slightly hyperosmotic transport of fluid.

According to the investigation by Sauer and Hair (1972) on the quantity of blood ingested by the lone star ticks which were fed on deer rather than sheep, we can estimate the relative importance of the salivary glands as a route of ion excretion (Table IV). On the assumption that water loss via the anus and integument is negligible during feeding, it is calculated that with an average net weight gain of 500 mg, 0.24 ml of fluid is excreted via the saliva from the total imbibition of 0.74 ml bloodmeal.

The data show that most of the sodium and chloride can be accounted for by elimination via the saliva, which is in agreement with our postulation of net movements of sodium and chloride ions in the bloodmeal across the gut epithelium from the gut lumen to the haemolymph with subsequent excretion of excess sodium and chloride via the saliva to prevent ionic imbalance. It is not surprising that the correspondence of potassium is not good as calculated from the whole sheep blood in which the potassium concentration is lower than that in the blood cells (erythrocytes in particular) (Altaman, 1961) (Table I). Nevertheless, the figures indicate that a relatively large portion of potassium ingested in the bloodmeal is retained in the tick; and by comparing the potassium concentration in different compartments of the feeding lone star tick, it is obvious that most of the potassium is stored in the gut lumen.

It should be mentioned that the ionic concentrations and freezing point depressions in the saliva collected from feeding lone star ticks using adrenaline as a stimulant of salivary secretion in the present study and three other methods (i.e., infra-red heat, pilocarpine, and electrical shock) by Barker et al. (1973) show great variation (Table V).

TABLE IV

AN ESTIMATE OF PERCENT SODIUM, POTASSIUM, AND CHLORIDE LOSS VIA THE SALIVA AND ANUS IN THE ADULT FEMALE LONE STAR TICK THROUGHOUT THE FEEDING PROCESS

	Content (mequiv. x 10^{-6})*				
Substance	0.74 ml Bloodmeal	0.24 ml Saliva	500 mg Tick	% of loss via the saliva	% of loss via the anus
Na^+	81400	46992	31050	93	7
K^+	23088	2904	21700	??	?
Cl^-	70374	36432	32800	97	3

* From Sauer and Hair (1972).

TABLE V

FREEZING POINT DEPRESSION AND IONIC CONCENTRATIONS IN THE SALIVA OF ADULT FEMALE FEEDING LONE STAR TICKS BY DIFFERENT METHODS OF STIMULATION

Stimulation method	Freezing point depression (-Δ°C)	Concentration (mequiv./l)		
		Na^+	K^+	Cl^-
Adrenaline	-0.68	200	15	150
Infrared heat*	-1.01	85	16	297
Pilocarpine*	-0.89	430	121	215
Electrical shock*	-0.93	218	49	196

* From Barker et al. (1973).

The osmolarities and ion compositions of the saliva obtained by the latter three stimulating methods bear little relationship with those in the haemolymph. Since the standing osmotic gradient hypothesis of fluid movement requires a close relationship between saliva and haemolymph composition, the use of adrenaline may be a more natural method for stimulating salivary secretion in feeding ixodid ticks.

Finally, we may add that Araman and Said (1972) have investigated the ionic concentrations in different compartments of two species of feeding argasid ticks, Argas (P.) persicus (Oken) and A. (P.) arboreus Kaiser, Hoogstraal and Kohls. The distribution of sodium, potassium, and chloride in the gut fluid and haemolymph in these two species follows the same pattern as that in the feeding lone star tick, which suggests that the mechanisms of movements of electrolytes and water across the gut epithelium are possibly similar to that of the ixodid ticks. Moreover, by comparing the ionic concentrations in the haemolymph and coxal glands in the two argasid ticks, one may assume that the coxal glands play a secretory function similar to the salivary glands of the ixodid ticks. Further experiments are needed to clarify these postulations between these two families.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This study describes the changes in the total body water content percent, osmolarities of the haemolymph and saliva, and the concentrations of sodium, potassium, and chloride in the whole tick, gut lumen, haemolymph, and saliva of the adult female lone star tick, Amblyomma americanum (L.) throughout the feeding process.

The water content percent increased sharply to 69.0 (from 52.8 in unfed ticks) at the beginning of the bloodmeal. It then decreased to 63.3 with the onset of rapid engorgement and decreased further to 57.5 prior to and just after completion of engorgement.

It appears that the salivary secretion can regulate the whole body water content percent by eliminating excess water absorbed with the bloodmeal, especially during the last phases of the bloodsucking process.

The lone star tick can regulate its body electrolyte composition because the concentrations of sodium, potassium, and chloride in each compartment are maintained at constant levels throughout the feeding process.

The sodium concentration in the host blood was higher than in the gut fluid, but lower than in the haemolymph; the reverse held true for potassium. The sodium concentration in the saliva was about 10 percent higher than in the haemolymph, but the potassium concentration in the haemolymph and saliva indicated no significant difference. The pattern

of distribution of chloride was very similar to that of sodium in the different compartments.

The measured high concentrations of sodium and chloride in the haemolymph and saliva with respect to the host blood and gut fluid indicate that sodium coupled with chloride may be actively transported from the gut lumen into the haemolymph, with subsequent excretion of excess sodium and chloride via the saliva. In contrast, the results suggest that a relatively large proportion of the ingested potassium is retained by the tick and stored in the gut lumen.

The osmolarities, measured as freezing point depression ($-\Delta°C$), in the haemolymph and saliva were -0.67 and -0.68, respectively. There was no significant difference of osmotic pressure between the haemolymph and saliva. It appears that the coupled movements of sodium and chloride ions may be the driving force for water movements from the haemolymph into the saliva to form the isosmotic fluid.

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