

SHORT COMMUNICATION

**HYPEROSMOTIC ORAL FLUID SECRETION DURING ACTIVE
WATER VAPOUR ABSORPTION AND DURING
DESICCATION-INDUCED STORAGE-EXCRETION BY THE
UNFED FEMALE TICK *AMBLIOMMA AMERICANUM***

BY MARVIN D. SIGAL^{1,*}, JOHN MACHIN² AND GLEN R. NEEDHAM¹

¹*Acarology Laboratory, Department of Entomology, The Ohio State University, Columbus, Ohio, USA* and ²*Department of Zoology, University of Toronto, Toronto, Canada*

Accepted 17 December 1990

It is well established that some acarines, insects and isopods maintain water balance in subsaturated air by actively absorbing water from the atmosphere (O'Donnell and Machin, 1988). Investigations of water balance physiology by Lees (1946) in the Ixodoidea demonstrated that partially desiccated ticks gain weight in water vapour activities (a_v = relative humidity/100) significantly lower than that of the haemolymph activities ($a_v \geq 0.98$). Subsequent research confirmed that active water vapour uptake is a common phenomenon in ticks (for reviews, see Rudolph and Knülle, 1978; Needham and Teel, 1986). The gnathosomal region was identified as the site of water vapour uptake when Rudolph and Knülle (1974) blocked various body surfaces of predesiccated adult *Amblyomma variegatum* with paraffin wax and monitored weight changes during exposure to a subsaturated a_v . A net weight gain occurred except when the gnathosoma was occluded. The external gnathosoma of the ixodid tick projects anteriorly from the basis capitulum as an assemblage of five processes (Fig. 1A). The most lateral appendages are a pair of palps that can be abducted from the inner dorsal chelicerae during attachment and blood feeding. The paired burrowing chelicerae and a singular ventral hypostome secure the oral sucking apparatus into the host feeding lesion during haematophagy. Wax-occlusion techniques have identified the proximal hypostome–cheliceral junction as a more specific site for vapour uptake in *A. americanum* (L.); and a correlation was found between palp abduction splaying behaviour and the critical equilibrium activity at which ticks were held (CEA) (M. D. Sigal, in preparation).

The exact mechanism of active water absorption in ticks remains undetermined

* Present address: Department of Life and Earth Science, Otterbein College, Westerville, OH 43081, USA.

Key words: active water vapour absorption, storage–excretion, *Amblyomma americanum*, Acari.

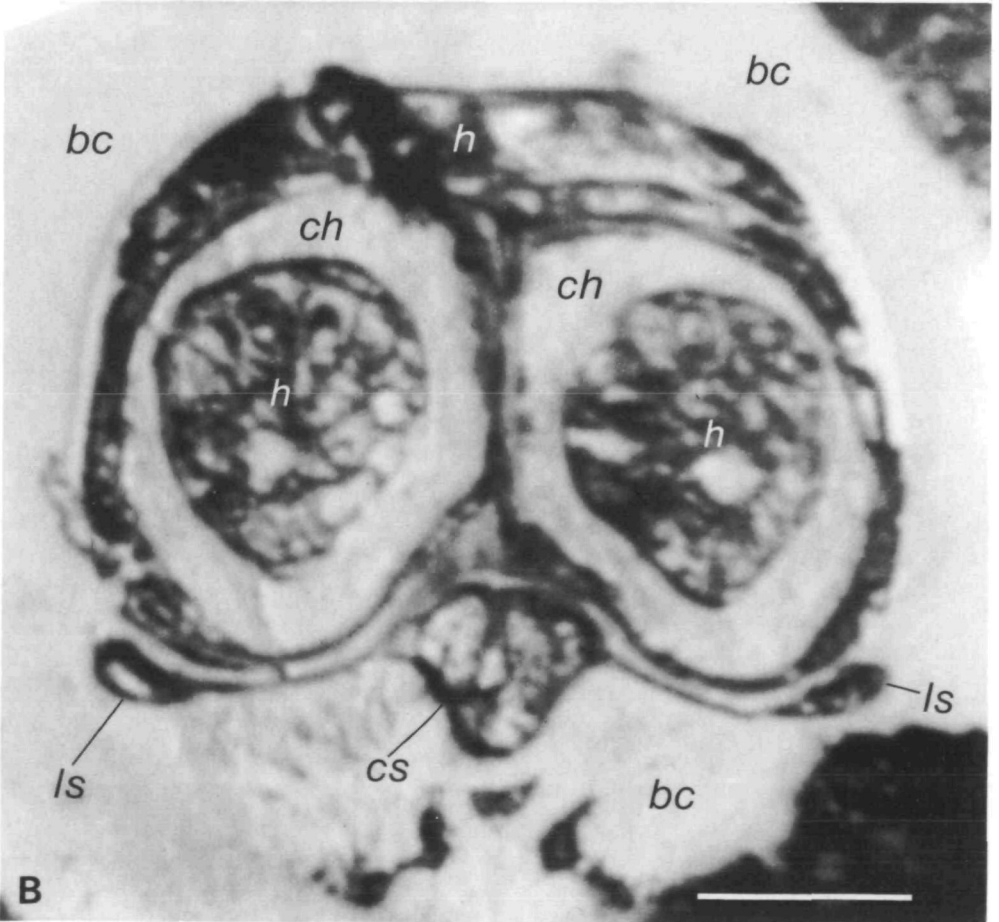
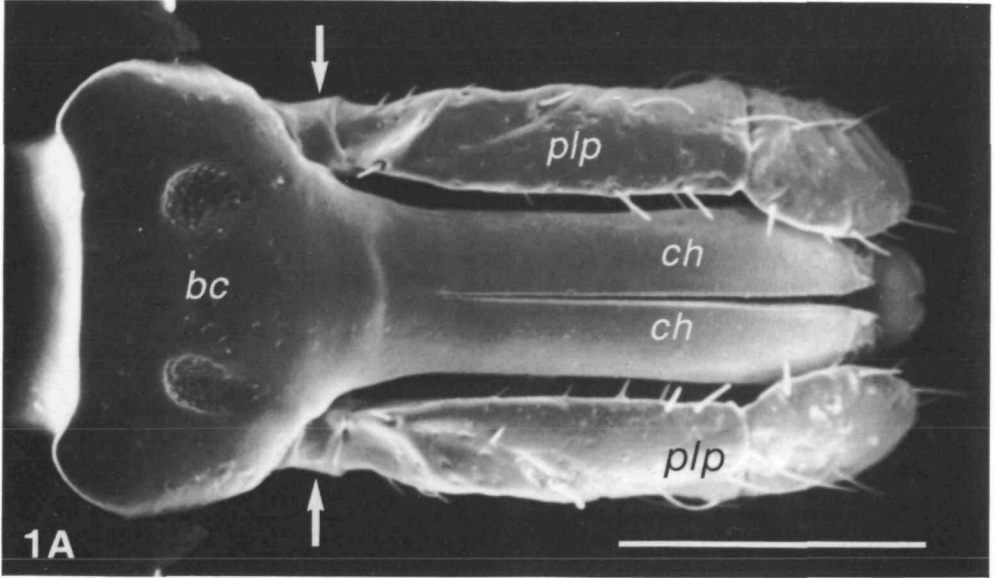


Fig. 1. (A) Scanning electron micrograph of the dorsal gnathosoma of an adult *Amblyomma americanum* from directly above. The white arrows indicate the approximate position of sections taken for melting-point analysis. Scale bar, 500 μm . (B) Frozen transverse section photographed at -9.0°C , dorsal side uppermost. The chelicerae (*ch*) containing haemolymph (*h*) and the central (*cs*) and lateral salivarium compartments (*ls*) containing oral fluid are clearly visible in the photomicrograph. The palps (*plp*) visible in the electron micrograph are not included in the transverse section. Pale material is cuticle of the basis capitulum (*bc*) and the chelicerae. The dark material in the central portion of the chelicerae as well as surrounding the chelicerae is frozen haemolymph, and the remaining dark material in the upper and lower right corner is frozen embedding compound. Scale bar, 200 μm .

(Needham and Teel, 1986). O'Donnell and Machin (1988) review several mechanisms that are involved in active water uptake, the most common of which is based on the colligative properties of a secreted, hyperosmotic material that can drive water uptake. Previous research on severely desiccated ticks has shown that Na^+ , K^+ and Cl^- salts are found on and in the mouthparts (Rudolph and Knülle, 1978; Devine, 1982). These deposited salts have been observed to deliquesce at a_v values near the CEA for the tropical bont tick *A. variegatum* (Rudolph and Knülle, 1978). However, it remains to be demonstrated whether more water is gained from the salt residues than is required to deliver them onto the gnathosoma. Needham and Teel (1986) suggest that desiccation-induced oral secretions in these long-lived ectoparasites may represent a storage-excretion mechanism to reduce elevated haemolymph osmotic pressures (OP) incurred by desiccation, with the advantage that these salts are reclaimed later during rehydration activity. Rudolph and Knülle (1978) used the protozoan parasite *Theileria* to destroy only types II and III acini of the salivary glands without preventing active uptake, and thereby indirectly implicated the type I agranular acini. Changes in the conformation of mitochondria demonstrated in these salivary acini during rehydration further suggest that the salivary glands are involved in vapour uptake (Needham *et al.* 1990).

This study employed nano-osmometry and melting-point analysis of frozen sections to measure the osmotic pressure of oral fluid of frozen sections and haemolymph in individual ticks in differing physiological states. Fluid osmolalities exceeding $5.49 \text{ osmol kg}^{-1}$, which would be equal to or greater than the equilibrium vapour activity of 0.91 a_v for unfed female *A. americanum* (Sigal, 1990), could function as the basis of a solute-driven, water vapour absorption mechanism. Unfed adult females of the ixodid tick *Amblyomma americanum* ($\text{CEA} \geq 0.88 a_v$; Sigal, 1990) were used in this study. Ticks were obtained from a laboratory colony reared at the Acarology Laboratory, The Ohio State University, Columbus, Ohio, maintained at 0.93 a_v and room temperature (26°C). Several 1-month-old female *A. americanum* were randomly selected from a cohort of ticks that had previously been fed on an adult chicken and used to induce oral fluid secretion as described below. Another group of ticks was also selected and placed in dry air for at least 24 h prior to rehydration and analysis of vapour uptake.

In the first fluid collection method we used light and heat stress to induce fluid production after the method of Barker *et al.* (1973). Ticks secured on a glass slide ventral side up with double-sided sticky tape were observed to exude fluid droplets laterally from the hypostome–cheliceral margin during high-intensity illumination with an incandescent microscope illuminator. Oral fluid samples (0.33–24.00 nl) were collected as soon as they were released by a finely drawn, oil-filled glass capillary inserted down the oral channel. Slight negative pressure was applied to the capillary through a 5 ml oil-filled syringe, connected by flexible polyethylene tubing. After the oral fluid had been collected and measured, a sample of haemolymph was similarly collected from the cut distal segment of leg I or II. Oral fluid samples and haemolymph droplets were injected under oil into the sample wells of a nanolitre osmometer (Clifton Technical Physics, Wanamassa, New Jersey). Melting points of up to four replicate unknowns were compared with those of two standard NaCl solutions either side of the expected range.

In the second method, oral fluid and haemolymph OP values were measured *in situ* in serially sectioned mouthparts. Prior to analysis, we established that ticks were actively absorbing water vapour by monitoring changes in water mass during rehydration using a Sartorius 4410 recording electrobalance ($\pm 1 \mu\text{g}$) operating in an airflow-regulated atmosphere at a_v values between 0.80 to 0.96 (Machin and Lampert, 1987). When mass gain was confirmed, the tick was quickly removed from the balance, quick-frozen in hexane cooled to near-freezing by liquid nitrogen and embedded in Tissue-tek embedding medium (Miles Inc., Elkhart, Indiana) on a microtome stub in a cryostat at -20.0°C . Transverse gnathosomal sections ($12 \mu\text{m}$) of the frozen mouthparts were placed on a slide in a droplet of chilled kerosene under a cover slip and inserted into a Mettler temperature-controlled stage (temperature resolution of $0.1^\circ\text{C}=0.054 \text{ osmol kg}^{-1}$) on a compound microscope positioned within the cryostat (Machin, 1979). Melting temperatures of the cheliceral haemolymph and the fluid of salivarium compartments were recorded by direct observation and from photographs taken as the sections were warmed from -20.0°C to approximately 0.0°C in $0.5\text{--}2.0^\circ\text{C}$ increments. The distinct lateral and central compartments of the salivarium, the two separate cheliceral haemolymph compartments and the surrounding haemolymph-filled basis capitulum were identified (Fig. 1B), and the crystalline ice structures of frozen fluids carefully noted. Observations were then made of changes in light refractive qualities and the appearance of liquified curvature of crystal edges during the graded series of intermittent temperature increases. Because of the extremely small size of the lateral compartments, the detection of crystalline particle movement was a useful criterion of melting, since it was difficult to say otherwise whether a single ice crystal in such a small compartment had melted.

Of the 11 ticks that yielded reliable data, seven comparisons between oral fluid and haemolymph osmotic pressures in the same individual were possible. Five of these showed considerably higher oral fluid OP values compared with corresponding haemolymph values. The remaining unpaired measurements were consistent with this pattern. In the light/heat-induced group (Table 1), oral fluid OP was

Table 1. The osmotic pressures (osmol kg^{-1}) and volumes (nl) of oral fluid stimulated by the desiccation-induced heat-lamp method, and haemolymph taken from five hydrated ($0.93 a_v$ *) and three desiccated ($\approx 0.0 a_v$, for 4 days) 1-month-old unfed adult female *Amblyomma americanum* (Ixodidae)

Tick number and condition	Fluid volume (nl)	Oral fluid (osmol kg^{-1})	a_w †	Haemolymph (osmol kg^{-1})	a_w †
Hydrated	1	3.69	0.43	0.992	—
	2	2.54	1.14	0.980	0.99
	3	24.00	0.44	0.992	0.23
	4	0.63	4.88	0.919	—
	5	—	5.11	0.916	—
Desiccated	6	1.14	4.57	0.924	0.87
	7	19.35	0.50	0.991	0.84
	8	2.46	1.96	0.966	0.79

* a_v , water vapour activity of the atmosphere.

† a_w , body fluid water activity. The activity of water vapour, a_v , in ambient air is calculated as percentage relative humidity/100 and is equal to a_w if the vapour is in equilibrium with a fluid at constant pressure and temperature. [See Arlian and Veselica (1979) for interconversions of colligative properties.]

quite variable, either considerably exceeding the more stable haemolymph values or resembling them. The sample sizes were too small to establish differences between hydrated and desiccated animals.

The OP values for some of the thermally induced oral fluid collected during this study were similar to the values simultaneously determined for haemolymph. It should be noted that the lowest OP values from oral fluid were obtained from the largest fluid volumes collected. This inverse correlation may be attributable to mixing with larger volumes of more dilute haemolymph resulting from the inadvertent rupturing of the delicate subchelicerale membrane sheath during insertion of the capillary. In some cases, when the capillary was inserted it was seen to be broken, with the tip lodged in the internal mouthparts of the tick. In these cases, the values were discarded. Nevertheless, the fluid collected may have been mixed with haemolymph following undetected injury, and could account for those OP values that were similar to those of haemolymph. Haemolymph OP values of hydrated ticks in our study were higher than in past determinations (Hsu and Sauer, 1975).

Melting-point analysis of oral fluid from the gnathosoma of rehydrating ticks shows that three compartments associated with the buccal region contained fluid with elevated OP values (Table 2). These were the two lateral compartments of the salivarium which communicate with the third central lumen compartment of the salivarium lying dorsal and anterior to the pharyngeal opening. The sections observed in this series were posterior to the divergence of the hypostome and chelicerae (Fig. 1A). The lateral compartments are seen in the photomicrograph

Table 2. Colligative properties associated with specific compartments of the salivarium and haemolymph in individual rehydrating female *Amblyomma americanum* ticks

Tick number	Lateral salivarium			Central salivarium			Haemolymph		
	Δt (°C)	(osmol kg ⁻¹)	a_w	Δt (°C)	(osmol kg ⁻¹)	a_w	Δt (°C)	(osmol kg ⁻¹)	a_w
1	12.0*	6.45	0.896	9.0	4.84	0.920	2.0	1.08	0.981
1	10.0†	5.38	0.912	5.0	2.69	0.954	1.0	0.54	0.990
2	—	—	—	5.0	2.69	0.954	2.0	1.08	0.981
2	—	—	—	—	—	—	1.0	0.54	0.990
3	—	—	—	—	—	—	3.0	1.61	0.972

The freezing point depression (Δt) was determined for collected body fluids (see text) as the lowest temperature at which the first ice crystals are observed to melt. These values were interconverted to osmolality (osmol kg⁻¹) or fluid vapour activity (a_w), as follows; $\Delta t = 1.858$ (osmol kg⁻¹), $a_w = 55.508 / (55.508 + \text{osmol kg}^{-1})$ and $\text{osmol kg}^{-1} = (55.508 - 55.508 a_w) / a_w$ (see Arlian and Veselica, 1979).

* Freezing point depression of the right lateral salivarium compartment from the first frozen section.

† Freezing point depression of oral fluid from the left lateral salivarium taken from the second frozen section.

of Fig. 1B. This interpretation was based upon the gnathosomal morphology of *Dermacentor andersoni* (Gregson, 1960) and *Hyalomma asiaticum* (Balashov, 1968) and confirmed by our serial sectioning of *A. americanum* mouthparts. The greatest fluid OP observed was 6.45 osmol kg⁻¹ (0.896 a_w) in the right lateral compartment of the salivarium (Table 2), while another high OP of 5.38 osmol kg⁻¹ (0.912 a_w) was observed in the left lateral compartment of a separate frozen section. Fluid OP in the central compartment below the chelicerae was 4.84 osmol kg⁻¹ (0.920 a_w). It should be noted that the fluids from tick number 1 were analyzed from an animal that had been exposed to a rehydrating period of over 16 h, which may account for the lower OP values observed in the central compartment. We suggest that the lower OP values (higher a_w values) of the central salivarium fluid represent fluid that had already been diluted by ambient water vapour and is rehydrated oral fluid which could be swallowed for net water gain. Since the size of the compartments observed were extremely small, the differences in the OPs of similar compartments may be due to variations in the *in situ* visual detection of the first ice crystal melting.

These results represent the first analysis of oral fluid from the gnathosoma of a rehydrating tick. The oral fluid is sufficiently hyperosmotic to absorb water vapour from a_w values reported within the CEA range for unfed, adult females of this species (Sigal, 1990). The OP values are among the highest for any fluid involved in active water vapour absorption in arthropods (O'Donnell and Machin, 1988). The OP of desiccation (heat)-induced oral fluid was also determined, and it indicates a highly concentrated fluid that may serve as a mechanism of storage-excretion for severely desiccated ticks. We conclude that active water uptake by adult *A. americanum* is a solute-driven process that is dependent upon the

production of a hyperosmotic fluid probably by type I agranular acini of the salivary glands.

This work was supported in part by research grants to Professor John Machin from the Natural Sciences and Engineering Research Council of Canada. We acknowledge Bernie Sigal, C. Cihon, C. Harless and R. Kazee of Chroma Studios, Columbus, Ohio, for their phototechnical support. We thank Dr Olaf Kahl for suggestions on a draft of the manuscript and, in particular, Dr Jonathan Wright for his valuable assistance and comments on the manuscript.

References

- ARLIAN, L. G. AND VESELICA, M. M. (1979). Water balance in insects and mites. *Comp. Biochem. Physiol.* **64A**, 191–200.
- BALASHOV, YU. S. (1968). Bloodsucking ticks (Ixodoidea) – Vectors of diseases of man and animals. Nauka, Leningrad. Translation in *Misc. Publ. Ent. Soc. Am.* (1972), **8**, 161–376.
- BARKER, R. W., BURRIS, E., SAUER, J. R. AND HAIR, J. A. (1973). Composition of tick oral secretions obtained by three different collection methods. *J. med. Ent.* **10**, 198–201.
- DEVINE, T. (1982). Neutron activation analysis of saliva from the tick *Amblyomma variegatum*. *Experientia* **38**, 676–677.
- GREGSON, J. D. (1960). Morphology and functioning of the mouthparts of *Dermacentor andersoni* Stiles. *Acta Tropica* **17**, 47–79.
- HSU, M. H. AND SAUER, J. R. (1975). Ion and water balance in the feeding lone star tick. *Comp. Biochem. Physiol.* **52A**, 269–276.
- LEES, A. D. (1946). The water balance in *Ixodes ricinus* L. and certain other species of ticks. *Parasitology* **37**, 1–20.
- MACHIN, J. (1979). Compartmental osmotic pressures in the rectal complex of *Tenebrio* larvae: evidence for a single tubular pumping site. *J. exp. Biol.* **82**, 123–137.
- MACHIN, J. AND LAMPERT, G. J. (1987). An improved water content model for *Periplaneta* cuticle: effects of epidermis removal and cuticle damage. *J. Insect Physiol.* **33**, 647–655.
- McMULLEN, H. L., SAUER, J. R. AND BURTON, R. L. (1976). Possible role of uptake of water vapour by ixodid tick salivary glands. *J. Insect Physiol.* **22**, 1281–1285.
- NEEDHAM, G. R., ROSELL-DAVIS, R., GREENWALD, L. AND COONS, L. B. (1990). Ultrastructure of type I acini in four species of ticks and the influence of hydration states on type I acini of *Amblyomma americanum*. *J. exp. appl. Acarol.* **10** (in press).
- NEEDHAM, G. R. AND TEEL, P. D. (1986). Water balance by ticks between bloodmeals. In *Morphology, Physiology, and Behavioral Biology of Ticks* (ed. J. R. Sauer and J. A. Hair), pp. 100–151. Chichester: Ellis Horwood Ltd.
- O'DONNELL, M. J. AND MACHIN, J. (1988). Water vapor absorption by terrestrial organisms. *Adv. comp. environ. Physiol.* **2**, 47–90.
- RUDOLPH, D. AND KNÜLLE, W. (1974). Site and mechanism of water vapour uptake from the atmosphere in ixodid ticks. *Nature* **249**, 84–85.
- RUDOLPH, D. AND KNÜLLE, W. (1978). Uptake of water vapour from the air: process, site and mechanism in ticks. In *Comparative Physiology; Water, Ions and Fluid Mechanics* (ed. K. Schmidt-Nielsen, L. Bolis and S. H. P. Maddrell), pp. 97–113. Cambridge: Cambridge University Press.
- SIGAL, M. D. (1990). The water balance physiology of the lone star tick, *Amblyomma americanum* (Acari: Ixodoidea), with ecophysiological comparisons to other Ixodid species. PhD dissertation. Columbus: The Ohio State University. 181pp.