ION AND WATER BALANCE IN THE IXODID TICK

DERMACENTOR ANDERSONI

I. ROUTES OF ION AND WATER EXCRETION

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INTRODUCTION

Terrestrial blood-sucking arthropods concentrate the nutrient portion of the blood meal by selective elimination of excess water. It has long been established that the Malpighian tubules are largely responsible for this water elimination in insects (Wigglesworth, 1931; Ramsay, 1955; Maddrell, 1964a,b). The argasid ticks excrete excess water via a pair of coxal glands (Bone, 1943), but until recently (Gregson, 1967; Tatchell, 1967b) the main excretory route employed by ixodid ticks was problematical.

A major contribution to our understanding of water-balance mechanisms in ixodid ticks comes from the work of Lees (1946, 1947). He suggested for Ixodes ricinus (1946) that excess fluid of the blood meal might be excreted by evaporation from the integument, although Lees himself recognized some shortcomings of this hypothesis (e.g. ticks feeding in a saturated or near-saturated micro-environment were still able to regulate body water content). A possible answer to this problem was suggested by Gregson (1967), who proposed that the salivary glands may function in osmoregulation. Tatchell (1967b) substantiated Gregson’s suggestion by demonstrating: (1) that the estimated total water loss from engorging Boophilus microplus was far greater than that measured through the integument, and (2) that tritiated water injected into the haemocoel of engorging ticks could be recovered from the blood and urine of the host. Tatchell inferred from the latter that the tracer could only have entered the host via the salivary glands. Belozerov (1967) also implicated the salivary gland as an important route for water excretion in two ixodid species (Ixodes ricinus and Dermacentor marginatus) largely on the basis of cuticular water-loss studies and the observation that the salivary glands increase in size during the progression of the feeding cycle. He points out that precedent has been set for such an hypothesis, since blood-sucking gamasid mites excrete water from the salivary glands after feeding (Belozerov, 1958).

Although to date the ‘salivary gland hypothesis’ has been reasonably supported in one species of ixodid tick (Boophilus microplus; Tatchell, 1967b, 1969), the latter species cannot be considered ‘typical’ of the family. First, it is a one-host tick (remaining on a single host throughout the larval, nymphal and adult feeds); secondly,
and more important, it does not discharge fluid from the rectal sac throughout the feeding cycle (Seifert, Springell & Tatchell, 1968). Other ixodid species, such as *Dermacentor andersoni*, defaecate considerably during the feeding period, and so one must consider the possibility that a sizeable proportion of the total water loss may be excreted via this route. It was primarily for this reason that the relative importance of the integument, anus and salivary glands as sites of water loss was re-assessed in *D. andersoni*. We also hoped to estimate the relative importance of the salivary glands and the anus in eliminating monovalent ions.

**MATERIALS AND METHODS**

All experiments were conducted on adult female *Dermacentor andersoni* taken from a laboratory culture.

**Rearing methods**

About 2 weeks after hatching, larvae were confined to the ear and scalp region of a rabbit by means of a cloth sac taped to the head. The larvae became engorged within 4 or 5 days and moulted into nymphs about 2 weeks later when kept at room temperature (21–25 °C) over saturated KNO₃ (relative humidity (r.h.) = 98% ; O'Brien, 1948).

Two-week-old nymphs were confined to the shaven back region of a rabbit by means of a foam rubber enclosure cemented to the skin. Nymphs engorged within 6 or 7 days and were stored at room temperature over saturated NaCl (r.h. = 88%) until they moulted into adults 3 or 4 weeks later.

The newly moulted adults were stored over saturated NaCl for 1 month, transferred to clean vials, and subsequently kept over saturated KNO₃ at 5 °C for 3–6 months. A few days prior to the adult feed the ticks were returned to room temperature and kept over saturated NaCl. As cautioned by Loomis (1961) only rabbits which had never before been infested with ticks were used for feeding adults.

**Water loss through the integument**

Ticks were allowed to commence feeding and at daily intervals a few were removed from the rabbit. The anal plates and mouthparts were plugged with a mixture of beeswax and resin (tacky wax). The ticks were weighed, returned to the rabbit, and re-weighed at 24 and 48 h. Thus these ticks were exposed to conditions that were identical in terms of relative humidity and temperature to those experienced by feeding ticks, but intake of blood and elimination of faeces were prevented by the wax plugs. The rate of integumentary water loss was taken as the rate of weight loss over the first 2 days following forced detachment from the host. This estimate includes metabolic weight loss and loss from the spiracles, genital orifice, and Gené's organ, none of which was covered with tacky wax.

**Collection of dry faeces**

Six polyethylene cylinders (3 cm diameter and 3 cm high) were glued to the back of a rabbit with epoxy resin. The area of skin surrounded by the cylinder was sprayed with a plastic surgical dressing ('Aeroplast', Parke Davis and Co.). This spray-on film prevented the tick's faeces from coming into direct contact with the salts associated
Ion and water balance in the ixodid tick. I

with the rabbit’s skin yet did not prevent the tick from attaching. Single females were placed in each of five capsules and five males were placed in the sixth. Dried faeces were collected from each capsule daily and were stored in separate polyethylene vials. On the sixth day of attachment the males were removed from their capsule and each was presented to a female for mating. The anal plate of the male was plugged with epoxy glue to prevent any of its faeces from being tallied with those of the female.

Determination of iron concentration

Haemoglobin concentration was calculated by determining the iron content of each sample by the method of Breuer & Militzer (1938), and by converting this value to haemoglobin content on the assumption that, by weight, 0.34% of haemoglobin is iron (Lemberg & Legge, 1949). Although Breuer & Militzer’s method does include a wet-ashing step in the procedure, this was not always sufficient to eliminate turbidity from the final solutions when using some of the tick samples. Therefore all samples were first dried in small platinum boats at 100 °C and then ashed at 460 °C for 5 h in a muffle furnace before carrying out the iron determinations. Although the recovery of iron from ashed inorganic standards was 98%, the recovery from ashed rabbit haemoglobin was only about 80%. Values from subsequent organic samples were corrected accordingly. Iron concentrations were read at 480 m\(\mu\) on a Unicam SP 500 spectrophotometer.

Procedure for sampling haemolymph and saliva

Haemolymph was taken from a severed leg segment under a stereomicroscope which was housed in a moist chamber kept near 100% R.H. This limited evaporation of haemolymph from the open wound during the time required for collection.

Saliva was collected in a glass capillary placed over the chelicerae and hypostome of the tick in the manner described by Howell (1966) and Tatchell (1967 a), except that no pharmacological stimulant was used to enhance the flow of saliva. Before placing the glass capillary tube over the mouthparts the oral end of the tube was dipped in liquid paraffin; in this way the column of saliva released into the tube was protected from evaporation. The total time for collection of saliva from a tick was 5–10 min.

The collected haemolymph and saliva were kept under liquid paraffin until analysed.

Determination of ion concentration and osmotic pressure

Sodium and potassium were determined by emission flame spectrophotometry with a Unicam SP 900 or a Techtron AA 120 flame spectrophotometer. Sodium samples were dissolved in distilled water and potassium samples in a 500 ppm Na (as NaCl) swamp solution. Homogenates of whole ticks or tick faeces were dry-ashed, but samples of rabbit blood, tick haemolymph and saliva were untreated before diluting in the distilled water or sodium swamp. Chloride concentration and osmotic pressure of haemolymph and saliva were determined by the first electrometric titration method of Ramsay, Brown & Croghan (1955), and the cryoscopic method of Ramsay (1949) respectively.
Haemolymph volume

Haemolymph volume was estimated using the tracer-dilution method. Inulin-carboxyl-14C (50 μC/25 mg; supplied by New England Nuclear) was dissolved in 5 ml of the tissue culture medium of Rehacek & Brzostowski (1969). The inulin solution was injected through a severed hind leg using an ‘Agla’ micrometer syringe (Burroughs Wellcome and Co.). The needle of the syringe was fitted to a fine-tapered glass pipette via a sleeve of PE tubing. The pipette was sealed within the leg-stump with tacky wax before injecting the tracer, and was left in place for several minutes after the injection. After removal of the pipette the wound was sealed with the tacky wax. Leakage from the leg was prevented during the whole procedure by the use at appropriate times of a fine bulldog clamp. The dosage for unfed ticks (approximately 10 mg weight) was 1 μl, and this was increased up to 3 μl for ticks weighing over 100 mg. All haemolymph samples (usually 1 μl) were added directly to Bray’s solution (Bray, 1960) for determination of 14C-activity using a Nuclear Chicago ‘Mark I’ liquid scintillation counter and the channels-ratio method for quench correction. The radioactivity of samples ranged between 10 and 60 times background. Since it was shown in several ticks that the radioactivity per unit volume of haemolymph was the same 1, 2 and 3 h after injection, haemolymph was thereafter sampled about 2 h following injection.

RESULTS

Total loss of fluid

In order to assess the relative importance of each potential route of excretion it was first necessary to determine the total quantity of fluid lost by ticks over the 7- to 10-day feeding period. Iron was considered a substance suitable for monitoring total imbibition since it (1) occurs exclusively in the meal (bound in porphyrin), (2) is not secreted by the salivary glands, and (3) is easy to assay. The volume of blood removed from the host was calculated from the total amount of haemoglobin imbibed and the measured haemoglobin concentration of whole rabbit blood (146 ± 11 mg/ml; mean ± S.E.). The total volume of fluid excreted by the tick was calculated using the formula

\[ W_f = M - (G + F_{dry}). \]

Where \( W_f \) is the total amount (mg) of fluid excreted during feeding, \( M \) is the total amount (mg) of meal imbibed, \( G \) is the net weight increase (mg) of the tick during feeding and \( F_{dry} \) is the dry weight (mg) of faeces passed by the tick during feeding.

Values for total intake and total excretion of four engorged females are present in Table 1.

The integument

Table 2 shows the integumentary water loss during a normal feeding period for 36 ticks separated into arbitrary weight ranges. Since the method used does not allow distinction between metabolic and evaporation losses, the estimates in Table 2 are probably maximal. Only about 40 mg of water were lost through the integument by the average adult female over a normal 7-day feeding period; this figure represents 2.5% at most of the total water loss (cf. Table 1).
Table 1. Intake and excretion of fluid by female ticks during the adult feeding cycle

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Total Hb removed from rabbit (mg)</th>
<th>Weight* of imbibed meal (mg)</th>
<th>Net weight increase of tick (mg)</th>
<th>Total weight of dry faeces (mg)</th>
<th>Total water loss (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>370</td>
<td>2530</td>
<td>600</td>
<td>288</td>
<td>1642</td>
</tr>
<tr>
<td>2</td>
<td>843</td>
<td>5630</td>
<td>1016</td>
<td>651</td>
<td>3943</td>
</tr>
<tr>
<td>3</td>
<td>606</td>
<td>4165</td>
<td>875</td>
<td>380</td>
<td>2910</td>
</tr>
<tr>
<td>4</td>
<td>470</td>
<td>3220</td>
<td>767</td>
<td>311</td>
<td>2142</td>
</tr>
</tbody>
</table>

* Calculated from haemoglobin concentration of rabbit whole venous blood (146 ± 11 mg/ml; mean ± S.E.)

Table 2. Weight loss by thirty-six female ticks with mouth and anus blocked at various points (i.e. weights) within the adult feeding cycle

<table>
<thead>
<tr>
<th>Weight range of feeding ticks (mg)</th>
<th>Number of ticks</th>
<th>Approximate time that an average tick spends in the weight range (days)</th>
<th>Mean weight loss per day (mg ± S.E.)</th>
<th>Average weight loss for ticks in each range (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfed-20</td>
<td>7</td>
<td>1·5</td>
<td>0·45 ± 0·27</td>
<td>0·7</td>
</tr>
<tr>
<td>20-40</td>
<td>3</td>
<td>1</td>
<td>0·84 ± 0·09</td>
<td>0·8</td>
</tr>
<tr>
<td>40-100</td>
<td>12</td>
<td>1·5</td>
<td>5·91 ± 0·83</td>
<td>8·9</td>
</tr>
<tr>
<td>100-200</td>
<td>9</td>
<td>1</td>
<td>8·03 ± 1·23</td>
<td>8·0</td>
</tr>
<tr>
<td>200-repletion</td>
<td>5</td>
<td>2</td>
<td>10·45 ± 2·64</td>
<td>20·9</td>
</tr>
<tr>
<td>Totals</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>39·3</td>
</tr>
</tbody>
</table>

Water loss from the anus and salivary glands

Differentiating between the quantities of fluid lost via the anus and salivary glands proved to be difficult. Since the excreted saliva is injected back into the host tissues throughout most of the feeding period, it was not feasible to collect it and measure its volume directly. All attempts to collect wet faeces and measure their water content before evaporation occurred were also unsuccessful. It was finally decided to take advantage of information on salt concentrations in the fluids of the tick and host which permit one to calculate the probable fluid loss via the salivary glands. These calculations become possible because of the fortunate circumstance that once salivation has commenced the sodium concentration of saliva (161 ± 3 m-equiv./l; mean ± S.E.) collected in glass capillaries does not change with increasing size of tick (Fig. 1); the assumption was therefore made that saliva injected naturally into the host also showed little variation in sodium concentration with the phase of engorgement. The total amount of sodium ingested (Table 3 C) during the feeding period was calculated from the volume of imbibed blood (Table 1) and the measured sodium concentration of rabbit whole venous blood (101 ± 2 m-equiv./l; mean ± S.E.). The total amount of sodium lost in the saliva by each tick during the feeding period was calculated from the following:

\[
\text{total amount of sodium excreted in the saliva} = \text{total amount of ingested sodium (Table 3 C)}
\]

- net gain of sodium by the engorged tick (Table 3 A)
- amount of sodium excreted in the faeces (Table 3 B)
In order to estimate the net gain of sodium by the tick for the above calculation, the sodium content per mg unfed tick was determined (0.085 ± 0.007 μ-equiv.). The total volume of saliva secreted during the feeding period (Table 3E) could then finally be calculated knowing the amount of sodium excreted in the saliva, and the sodium concentration of the saliva. The actual data used in the above calculations are presented in Table 3, and show that the volume of salivary secretion accounts for 74 ± 1% (mean ± s.e.) of the total water lost during feeding.

Table 3. Calculation of salivary water loss

<table>
<thead>
<tr>
<th>Serial no. (as in Table 1)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Mean ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Net Na retained by engorged tick and its cuticle (μ-equiv.)</td>
<td>27.3</td>
<td>51.6</td>
<td>49.3</td>
<td>35.9</td>
<td>—</td>
</tr>
<tr>
<td>(B) Na content of dry faeces (μ-equiv.)</td>
<td>10.8</td>
<td>11.1</td>
<td>15.2</td>
<td>17.4</td>
<td>—</td>
</tr>
<tr>
<td>(C) Total Na ingested (μ-equiv.)</td>
<td>240</td>
<td>534</td>
<td>395</td>
<td>395</td>
<td>—</td>
</tr>
<tr>
<td>(D) Na lost via the salivary gland ('C'-'A'-'B')</td>
<td>202</td>
<td>471</td>
<td>330</td>
<td>252</td>
<td>—</td>
</tr>
<tr>
<td>(E) Volume* of saliva (μl)</td>
<td>1261</td>
<td>2943</td>
<td>2064</td>
<td>1574</td>
<td>—</td>
</tr>
<tr>
<td>(F) Saliva volume as percentage of total water loss</td>
<td>76.8</td>
<td>73.9</td>
<td>70.9</td>
<td>73.5</td>
<td>74.0 ± 1.2</td>
</tr>
</tbody>
</table>

* Volume calculated to remove the Na in 'D' assuming Na concentration of saliva = 161 m-equiv./l.

Table 4. The routes of excretion for sodium and potassium in the female tick during the adult feeding cycle

<table>
<thead>
<tr>
<th>Serial no. (as in previous tables)</th>
<th>(1) Total ingested (μ-equiv.)</th>
<th>(2) Total excreted (μ-equiv.)</th>
<th>(3) Faecal content (μ-equiv.)</th>
<th>(4) Saliva content ('2'-'3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>241</td>
<td>214</td>
<td>11</td>
<td>203</td>
</tr>
<tr>
<td>2</td>
<td>537</td>
<td>485</td>
<td>11</td>
<td>474</td>
</tr>
<tr>
<td>3</td>
<td>397</td>
<td>348</td>
<td>15</td>
<td>333</td>
</tr>
<tr>
<td>4</td>
<td>397</td>
<td>271</td>
<td>17</td>
<td>254</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>102</td>
<td>66</td>
<td>55</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>228</td>
<td>149</td>
<td>124</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>169</td>
<td>90</td>
<td>72</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>130</td>
<td>73</td>
<td>59</td>
<td>14</td>
</tr>
</tbody>
</table>

Routes of ion loss

With values for total meal intake of sodium and potassium, and for the concentrations of these ions in the saliva and faeces (Table 4), it was possible to estimate the relative importance of the salivary glands and anus in the excretion of sodium and potassium. One arrives at the following conclusions from Table 4: of the total ingested sodium and potassium, 89 and 60% were eliminated respectively. However, whereas 96% of the excreted sodium was lost in the saliva, only 18% of the excreted potassium was lost via this route. Conversely, 82% of the excreted potassium, but only 4% of the excreted sodium appeared in the faeces. The figures for chloride were not complete, but it appeared that this ion was excreted in a manner similar to that of sodium.
Ionic and osmotic changes in the haemolymph and saliva during a normal feeding period

In order to gain some insight as to the mechanism of fluid secretion by the salivary gland (see Kaufman & Phillips, 1973a, b), the ion concentrations of haemolymph and saliva were monitored throughout the feeding period (Fig. 1). Haemolymph of the unfed tick had a fairly high ionic content (280 m-equiv./l. Na), but this fell with the progression of feeding to about 160 m-equiv./l on the third day. This level was maintained until repletion. Likewise, the chloride concentration of haemolymph fell from 170 m-equiv./l initially, to 125 m-equiv./l after 5 days and then remained relatively constant. Similarly, potassium fell from about 20 to 7-5 m-equiv./l. Comparisons of

Fig. 1. Ion concentrations of haemolymph (open bars) and saliva (hatched bars) with time in the adult female during the feeding period. Vertical bars indicate s.e. of the means. The concentrations of sodium, chloride and potassium in the blood meal were 101, 89, and 43 m-equiv./l, respectively.
Fig. 2. Osmotic pressure of haemolymph and saliva with time in the adult female during the feeding period. Samples of haemolymph (○) and saliva (△) from the same tick are joined by straight lines. Although the osmotic pressure of whole rabbit blood was not measured cryoscopically, the osmotic pressure of human plasma is approximately 306 mOsm/l (Ruch & Patton, 1965).

Fig. 3. Volume of haemolymph in the adult female with the progression of feeding. Haemolymph volumes were determined from the dilution of injected 14C-inulin (see text). The regression curve shows the best straight line through the points \( Y = -0.55 + 0.23X \).
ion and water balance in the ixodid tick. I

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Fig. 4. Effect of injecting saline into the haemolymph on the subsequent volume of salivary secretion. Volume was determined by collecting the saliva in calibrated capillary tubes (see text). The percentage of haemolymph that this represented was calculated, assuming that 23% of the body weight plus the injected fluid equals haemolymph volume. ○, No injection; △, injection of 1-2% NaCl (1 µl/20 mg tick weight) before collecting saliva; ■, injections of 1-2% NaCl (1 µl/10 mg tick weight before salivation) after collecting saliva from some indi-

sodium and potassium concentrations in saliva and haemolymph of individual ticks indicated no significant differences; the chloride concentration of the saliva, however, was about 10% higher than that of the haemolymph (P = 0.01; pairs t-test) over the whole time course of salivation. Osmotic pressure (Fig. 2) of the haemolymph fell from 527 ± 21 mOsm/l (mean ± s.e.) on the first day of feeding and remained at 375 ± 6 mOsm/l through the remainder of the feeding period. The saliva was slightly, but consistently, hypo-osmotic (P = 0.02) to the haemolymph by about 5% (saliva = 356 ± 4 mOsm/l).

**Haemolymph volume**

In this study it was important to determine whether inulin could be secreted by the salivary glands or Malpighian tubules, or whether it could diffuse into a large compartment such as the gut and thus result in an overestimate of haemolymph volume. The following experiments showed that errors resulting from the latter possibilities were probably quite small. Six partially fed ticks were injected with large doses of 14C-inulin and then returned to the rabbit to recommence feeding, salivation, and defaecation. The next day freshly extruded faeces was collected, and haemolymph and saliva were sampled. There was no detectable 14C-activity in any of the six saliva samples, although the haemolymph contained 5000-40000 cpm/µl of radioactivity. Clearly inulin is not secreted by the salivary gland. The activity of the faeces at the
same time was 310 cpm/mg dry weight (mean value for faecal samples pooled from a number of injected ticks); thus inulin is only slowly eliminated from the haemolymph.

Haemolymph volume increased linearly with increasing weight of the tick, and always comprised about 23% of the body weight (Fig. 3). That the haemolymph volume is maintained at a constant proportion of the body weight (as the tick becomes engorged to 75 times its unfed weight) suggests that the volume of this compartment is under some control. We supposed that it is the act of salivation which ultimately regulates the volume of haemolymph, and therefore we tested whether artificially increasing haemolymph volume could stimulate or induce salivation into glass capillary tubes. However, compared to non-injected controls, salivation was inhibited when the haemolymph volume was increased by 25% or 50% with iso-osmotic saline (1.2% NaCl) (Fig. 4). One can coax a tick forcibly removed from a rabbit to secrete into a capillary tube and then restore its haemolymph volume by injections of saline; however, this treatment failed to induce salivation (Fig. 4).

**DISCUSSION**

A pictorial summary of the essential findings is presented in Fig. 5. The female tick excretes ions via both the anus and the salivary glands; however, most of the excreted sodium and probably chloride are lost in the saliva and most of the excreted

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**Fig. 5.** Summary of ingestion and elimination of ions and water by the female tick during a normal adult feeding cycle. Heavy solid arrows denote major routes. Fine solid arrows denote minor routes. Heavy broken arrow denotes a possible major route. Percentage figures refer to proportions of the total amount excreted over the complete feeding period. (a) Meal derived from a mixture of whole blood and other tissue fluids. (b) Na and water, but probably a lesser amount of K is transferred from the gut diverticula to the haemolymph. (c) Na (as NaCl), water and some K in excess of the tick's requirements are transferred back to the host in the copious salivary secretions. (d) A small quantity of water is evaporated through the integumentary surface. (e) Most of the potassium probably passes directly from the gut diverticula to the rectal sac and out through the anus, or alternatively (f) The possibility cannot be excluded that potassium enters the haemolymph and then is transferred to the faecal material via the Malpighian tubules.
potassium in the faeces. One explanation for the differing routes of excretion is that
the major portion of the faeces is derived from host blood passing directly into the
rectal sac from the midgut. If the midgut epithelium can transport sodium, chloride
and water into the haemolymph but, is relatively impermeable to potassium, this
would result in the observed high potassium and low sodium in the remains of the
meal entering the rectal sac. There is some evidence for this hypothesis. Once the
haemolymph has attained a stable composition 3 or 4 days after feeding has com-
menced, the sodium concentration in the haemolymph (160 m-equiv./l) is somewhat
higher than the sodium concentration in the meal (100 m-equiv./l). Similarly, the
chloride concentration in the haemolymph (125 m-equiv./l) is also higher than that in
the meal (90 m-equiv./l). However, haemolymph potassium (7.5 m-equiv./l) is con-
siderably less than that of the meal (42 m-equiv./l). These figures are consistent with
the transport of sodium and chloride across the gut epithelium in excess of potassium.
The relative impermeability of the gut epithelium to potassium has been suggested
for the argasid tick, Ornithodorus moubata (S. E. Kaufman, 1971). Alternatively, one
cannot rule out another plausible explanation. Since potassium is the major cation in
the Malpighian tubule secretion of several insects (Ramsay, 1953; Berridge, 1968;
Irvine, 1969; Maddrell, 1969; Pilcher, 1970) it might be absorbed from the midgut
and then rapidly secreted by the Malpighian tubules of the tick. Provided that re-
absorption of potassium in the rectal sac were lower than in insects studied to date,
the net result would be a low potassium concentration in the haemolymph and a high
concentration in the faeces. Although there is no clear-cut evidence which opposes
the latter mechanism, there are some facts available which make it less attractive than
the first explanation. On the basis of histological investigation, both Balashov (1958)
and Till (1961) report that the Malpighian tubules do not become very active until
after the tick detaches from the host. Most of the accumulation of guanine in the
Malpighian tubules and rectal sac occurs at that time and is probably due to meta-
bolism associated with egg development. This appears to be the situation in D. ande-
roni as well (unpublished observations). However, more direct evidence on the
quantity of fluid and potassium secreted by the Malpighian tubules during the feeding
period is clearly desirable.

On dry-weight basis the sodium concentration in male faeces was about six times
that in female faeces (217 m-equiv. Na/kg dry weight and 38 m-equiv. Na/kg dry
weight, respectively). This finding suggests that males eliminate salt and water
differently from females. Although there is little doubt now that the salivary glands
are important for water regulation in the female of two ixodid species, for a variety
of reasons it has not yet been necessary to postulate the same for the male. First,
growth of the salivary glands during feeding is not as marked in the male as in the
female (Till, 1961; Chinery, 1965). Secondly, the male imbibes only a modest amount
of blood, and so it is not faced with the task of excreting large volumes of excess fluid.
Finally, the paralytic factor (most probably carried in the saliva) is only rarely trans-
mitted by the male (Gregson, 1943). One would suspect that anal and integumentary
water loss might suffice to account for osmoregulation in the male.

When one compares the ionic composition of haemolymph and saliva from Derma-
centor andersoni and that from Boophilus microplus (Tatchell, 1969), some differences
emerge. In Dermacentor the saliva to haemolymph (S/H) ratio for sodium and potas-
sium is insignificantly different from one: in Boophilus, however, the S/H ratio for sodium is greater than one, and that for potassium less than one, although Tatchell does not state whether the differences are significant. In both species the S/H ratio for chloride is 1:1. The saliva of Boophilus is hyperosmotic to the haemolymph (S/H = 1:23), whereas in Dermacentor it is slightly (but significantly) hypo-osmotic to the haemolymph (S/H = 0:94). With the assumption that the primary secretion in Dermacentor is iso-osmotic or hyperosmotic to the haemolymph (i.e. that flow of fluid is driven by a local osmotic gradient), then reabsorption of solute relative to water may occur somewhere between the salivary acini and the oral cavity; micro-puncture studies on vertebrate salivary glands demonstrate clearly that the ducts are responsible for solute reabsorption and hence the elaboration of a hypotonic saliva (Martinez, Holzgreve & Frick, 1966; Mangos, Braun & Hamann, 1966; Young & Schögel, 1966). Such may also be the case for Dermacentor. Since in Boophilus the saliva is hyperosmotic (as expected for a secretory system), this would suggest that in the latter species, either the ducts serve merely as a delivery system for the saliva, or that they possibly secrete solute as well. With this in mind it would be interesting to compare the ultrastructure of the ducts in these two species.

Despite the large net flux of ions and water through the haemolymph compartment (in all, 9 to 12 times the haemolymph volume measured at repletion), the ratio of extracellular fluid to body weight remains constant throughout the feeding cycle (Fig. 3). This suggests that the rate of salivary secretion is correlated with fluid intake. Maddrell (1964c) concluded that the release of diuretic hormone (i.e. the stimulus to urine secretion by the Malpighian tubules) in Rhodnius is linked to fluid intake through abdominal distention via receptors in the tergo-ster nal muscles. We have not performed the experiments necessary to reveal stretch-receptors controlling salivary gland activity in Dermacentor; but at least salivary secretion does not appear to be related in a straightforward way to haemolymph volume (Fig. 4).

This paper provides evidence that the salivary gland is the major route whereby excess NaCl and water are excreted in Dermacentor, and implies that as a result of this process control over the volume of haemolymph may be exercised. The mechanism of salivation (whether fluid is produced by a secretory or a filtration-resorption process) and the control (whether by nerves or hormones) are examined in a subsequent paper (Kaufman & Phillips, 1973a).

**SUMMARY**

1. Of the total meal imbibed by female Dermacentor andersoni during the normal adult feeding cycle, about 80% is excreted. Of the total water excreted by the tick, 75% is removed by salivation, less than 3% is evaporated from the integument and spiracles, and the remainder is lost via the anus.

2. Of the total excreted sodium and potassium, 4 and 82% respectively are lost via the anus. The remainder in each case is presumed excreted via the salivary glands.

3. The ionic and osmotic concentrations of the haemolymph and saliva stabilize at constant values by the third or fourth day of feeding. The volume of extracellular fluid is constantly maintained at 23% of the body weight, even though the total body weight increases 75 times over the unfed weight, and the volume of excreted fluid
Ion and water balance in the ixodid tick. I

passing through the haemolymph is about ten times the haemolymph volume at repletion.

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