THE INFLUENCE OF VARIOUS FACTORS ON FLUID SECRETION BY IN VITRO SALIVARY GLANDS OF IXODID TICKS

By WILLIAM KAUFMAN

School of Veterinary Medicine, Madingley Road, Cambridge, England
Institut de Zoologie, Université de Neuchâtel, Neuchâtel, Switzerland*

(Received 11 November 1975)

SUMMARY

1. Salivary glands of the female ixodid tick, Dermacentor andersoni, secrete fluid in vitro when bathed in a slightly modified version of the mammalian tissue culture medium ‘TC 199’.

2. Rate of salivation in vitro increases with progression of feeding, but there is no comparable increase in dry weight of the salivary glands during the early phase of engorgement. Engorged ticks secreted at only 25% the rate of 90-250 mg ticks, indicating that salivary gland degeneration has already begun in the very early post-engorgement stage.

3. A salivary gland stimulating factor can be detected in the nervous system but not in other tissues.

4. Male salivary glands secrete at only 1/20th the rate of female glands. Thus males probably do not use their salivary glands as osmoregulatory organs.

5. From the uniform lack of response to ACh and uniform response to DA in 7 ixodid tick species, it is suggested that the control of salivation is similar throughout the ixodid family.

INTRODUCTION

It is presently believed that an important function of the salivary glands of female ixodid ticks is to regulate the internal volume and composition of body fluid during the blood-sucking period by secreting fluid into the host (Tatchell, 1967b; Kaufman & Phillips, 1973a). Partially or fully fed ticks can be induced to expel several microlitres of saliva by placing a glass capillary tube of specific bore over the mouthparts, but the total volume of secreted saliva can be increased approximately sixfold by previous injection of the cholinomimetic, pilocarpine (Tatchell, 1967a). Kaufman & Phillips (1973b) adopted a method for observing secretion of the salivary glands in vitro but found that cholinomimetics were incapable of eliciting fluid secretion under these conditions. However, the catecholamines, adrenaline (ADR), noradrenaline (NA) and dopamine (DA) were potent in vitro stimulants. Adrenaline was also used as an in vivo stimulant in concentrations far lower than those previously reported for pilocarpine. We thus advanced the hypothesis that the fluid secretory cells were controlled directly by an adrenergic agent and that cholinomimetics probably exerted

* Present address.
their *in vivo* effects indirectly. Finally, other evidence suggested that the natural salivary stimulant was not a haemolymph-borne factor (Kaufman & Phillips, 1973b).

In this paper I present an artificial medium that is far superior to the one used earlier, extend some observations to male salivary glands and to glands from a variety of ixodid ticks, and suggest the source of the natural salivary stimulant.

**MATERIALS AND METHODS**

*Experimental animals*

Ticks were taken from established laboratory colonies, except for specimens of *Ixodes ricinus* L., *I. hexagonus* Leach, and *Dermacentor reticulatus* Fabricius which were caught in the field. *Dermacentor andersoni* Stiles was reared as described earlier (Kaufman & Phillips, 1973a), those conditions being the most reliable for breaking adult diapause according to the studies of Wilkinson (1971).

*Ion determination*

Sodium and potassium ion concentrations of artificial media and secreted fluid were determined using standard techniques of flame emission spectrophotometry on a Pye Unicam SP 191 Atomic Absorption Spectrophotometer. Sodium samples were diluted in distilled water and potassium samples in a 3 m-equiv/l NaCl swamp solution. All samples and standard solutions were kept frozen in 'Nalgene' containers until measured.

*Artificial medium*

Glands were prepared for *in vitro* secretion in the manner described earlier (Kaufman & Phillips, 1973b). The artificial medium used was 'TC Medium 199' (Wellcome Reagents Ltd) with NaCl replacing the NaHCO₃; MOPS buffer (Calbiochem) was also added (10 mM/l) as were the antibiotics, Penicillin (200 units/ml) and streptomycin (100 units/ml). The pH was adjusted to 7.0 with NaOH and the medium was continuously oxygenated and held at 27 °C as described earlier (Kaufman & Phillips, 1973b). Although I shall refer to the medium hereafter as ‘TC 199’, one should bear in mind that it has been modified in the manner just described in order to avoid alkalinization which would otherwise have occurred. Tissues were excised mainly in ‘Dissection medium 2’ (Kaufman & Phillips, 1973c) but in later experiments I turned to ‘Hank’s Balanced Saline’. The composition of this saline in mg/l is: NaCl, 8000; Na₂HPO₄, 48; KCl, 400; KH₂PO₄, 60; CaCl₂, 140; MgSO₄, 98; d-glucose, 1600; phenol red, 10.

*Pharmacological agents*

Acetylcholine chloride, L-adrenaline, dopamine hydrochloride, theophylline and cyclic AMP were supplied by Sigma Chemicals Ltd. Stock solutions of each drug (10⁻³ M) were prepared fresh daily and were diluted to working strength by the use of disposable micro-volumetric capillary tubes.
Tissue homogenates

Ticks (90–300 mg) were allowed to secrete saliva into capillary tubes; after approximately 1 µl had been collected, the dorsum was slit with a razor blade scalpel and the exuded haemolymph collected (30–90 s in each case). Each sample was immediately heat-treated for 5 min at 60 °C and then placed on ice. The coagulum resulting from heat-treatment could not be separated from a ‘serum’ by centrifugation, and so the contents of each capillary tube were pushed out by means of a fine wire, and the pooled coagulum (about 100 µl from 15 ticks) was homogenized in 200 µl TC 199 before testing its stimulant activity on in vitro salivary glands. Synganglia, ovaries and Malpighian tubules were dissected out of partially fed ticks (110–330 mg), placed on ice and homogenized in the desired volume of TC 199 for testing salivary stimulant activity.

Wet and dry tissue weights

Unfed specimens of Amblyomma hebraeum Koch (chosen within a narrow weight range) were allowed to feed on rabbits and their salivary glands were dissected out under Hank's balanced saline at various stages of the feeding cycle. The glands were freed of all extraneous tissue. Extra-glandular fluid was removed by dabbing the tissue on filter paper according to a standard procedure and the glands were then placed on pre-weighed pieces of aluminum foil. Wet weight of each salivary gland pair was read on a Mettler M5 microbalance to within the nearest 2 µg, 90 s after removing the glands from the dissecting fluid. The tissue was then dried at 100 °C. Dry weights were found to be constant between 45 min and 17 h, so dry weights were recorded within this interval.

All data are presented in the following format: Mean ± s.e. (n). Where appropriate, statistically significant differences are indicated.

Results

The nutrient medium

Fig. 1 shows that in vitro glands secrete maximally in 10⁻⁶ M DA in TC 199, but glands bathed in Normal Ringer Solution (NRS; for composition see Kaufman & Phillips, 1973b) plus 10⁻⁶ M ADR secreted at only half that rate (Fig. 2). Although ADR can elicit only about 90 % of the maximum rate (Fig. 1), clearly this fact does not explain the discrepancy between the treatments compared in Fig. 2. In addition, the discrepancy remained when 10⁻⁶ M DA was used to stimulate secretion in both media (124 ± 16 nl min⁻¹, n = 4 in NRS and 228 ± 36 nl min⁻¹, n = 4 in TC 199). The overall rate of decline of secretion in TC 199 is similar to that in NRS (Fig. 2).

The saliva to medium ratio (s/m) for Na⁺ is similar in TC 199 (1.07) and in NRS (1.11), although the ratio for K⁺ is somewhat lower in TC 199 (1.19) than in NRS (2.12). Na⁺ and K⁺ concentrations of in vitro saliva are independent of both the weight of tick from which the glands were excised (Fig. 3) and of the prevailing rate of fluid secretion (Fig. 4). They are also independent of the pharmacological species used to stimulate secretion (of ADR, DA and ergometrine). Moreover, levels of Na⁺ and K⁺ in saliva secreted by glands held in organ culture remain constant for up to 7 days.
after excision. In summary, the ionic mechanism generating fluid secretion is much the same in TC 199 as observed previously in NRS (Kaufman & Phillips, 1973c). The half-maximum and maximum rates of secretion were attained much more rapidly in TC 199 to which $10^{-6}$ M DA had been added than in NRS (Table 1). Although in NRS the time to decay to the half-maximum rate after removing ADR was significantly less than the time to reach the same rate on addition of ADR, such was not the case for DA in TC 199.

In TC 199 the threshold concentration for ADR is about $5 \times 10^{-8}$ M and the maximum rate of secretion occurs in $5 \times 10^{-6}$ M (Fig. 1); the corresponding values in NRS were about $10^{-6}$ M and over $10^{-6}$ M (Kaufman & Phillips, 1973b).
Fluid secretion of ixodid ticks

$10^{-2}$ M cyclic AMP did not stimulate fluid secretion in NRS (Kaufman & Phillips, 1973b) but was able to do so in TC 199. Theophylline ($10^{-2}$ M) neither stimulated fluid secretion on its own nor potentiated the response to cyclic AMP; the response to cyclic AMP was thus, at best, only 20% of the maximum induced by $10^{-6}$ M DA. However, $10^{-2}$ M theophylline was able to potentiate the response to low concentrations ($5 \times 10^{-9}$ M) of DA. Lower concentrations of theophylline were ineffective, and potentiation did not occur when the concentration of DA was raised to $5 \times 10^{-8}$ M (Fig. 5).

It is possible that the presence in TC 199 of a surface active agent (Tween 80) might make the salivary glands generally more sensitive to pharmacological agents. In support of this it was shown that theophylline in NRS could induce a secretory rate that was at best only barely detectable, whereas 5 mg/l Tween 80 augmented the rate 16-fold, although secretory rate was still submaximal (Fig. 6). Tween 80 could not potentiate the action of $10^{-6}$ M DA in NRS even though these glands did secrete much more rapidly when transferred to TC 199 (p. 729). The results with cyclic AMP were not as encouraging since no potentiation with Tween 80 was observed (Fig. 6).

The role of the calcium ion in excitation–effect coupling is now well established

---

Figs. 3 and 4. Concentration of Na$^+$ and K$^+$ of *in vitro* saliva of *D. andersoni* as a function of tick weight (Fig. 3) and rate of fluid secretion (Fig. 4). The abscissae are in logarithmic scale. Open bar-diagrams represent mean and s.e. of the plotted points. Blackened bar-diagrams represent mean and s.e. for the bathing medium, TC 199. The differences between saliva and medium, though slight, are highly significant ($P < 0.01$). The slopes of the regression curves through the plotted points (not drawn in the figures) are all insignificantly different from zero ($P > 0.05$).
Table 1. Comparison between TC 199 and NRS in relation to various parameters of fluid secretion

<table>
<thead>
<tr>
<th>Parameter Description</th>
<th>NRS with 10^-4 M ADR</th>
<th>TC 199 with 10^-4 M DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>First exposure to drug</td>
<td>27 °C</td>
<td>27 °C</td>
</tr>
<tr>
<td>(A) Maximum rate of secretion (nl/min)</td>
<td>110 ± 10.5 (26) HS</td>
<td>234 ± 16 (12)</td>
</tr>
<tr>
<td>(B) Min to reach half-maximum rate on addition of drug</td>
<td>8.5 ± 1.0 (26) HS</td>
<td>4.6 ± 0.4 (12)</td>
</tr>
<tr>
<td>(C) Min to reach maximum rate on addition of drug</td>
<td>17.0 ± 1.6 (26) HS</td>
<td>82 ± 0.8 (12)</td>
</tr>
<tr>
<td>(D) Min to return to half-maximum rate after removing drug</td>
<td>2.3 ± 0.5 (8) S</td>
<td>4.0 ± 0.4 (12)</td>
</tr>
<tr>
<td>Second exposure to drug</td>
<td>27 °C</td>
<td>27 °C</td>
</tr>
<tr>
<td>(A)</td>
<td>52 ± 19 (7) HS</td>
<td>228 ± 12 (12)</td>
</tr>
<tr>
<td>(B)</td>
<td>8.5 ± 1.5 (7) HS</td>
<td>7.5 ± 0.2 (12)</td>
</tr>
<tr>
<td>(C)</td>
<td>17.0 ± 2.0 (7) HS</td>
<td>8.5 ± 0.1 (12)</td>
</tr>
<tr>
<td>(D)</td>
<td>3.8 ± 1.4 (5) NS</td>
<td>4.2 ± 0.6 (11)</td>
</tr>
<tr>
<td>Third exposure to drug</td>
<td>27 °C</td>
<td>27 °C</td>
</tr>
<tr>
<td>(A)</td>
<td>6.7 ± 1.1 (13) HS</td>
<td>174 ± 27 (10)</td>
</tr>
<tr>
<td>(B)</td>
<td>6.7 ± 0.9 (13) HS</td>
<td>3.7 ± 0.2 (10)</td>
</tr>
<tr>
<td>(C)</td>
<td>18.4 ± 1.2 (13) HS</td>
<td>9.6 ± 0.4 (10)</td>
</tr>
<tr>
<td>(D)</td>
<td>—</td>
<td>4.6 ± 0.6 (10)</td>
</tr>
</tbody>
</table>

* NS, Not significantly different (P > 0.05).
S, Significantly different (0.05 > P = 0.01).
HS, Highly significantly different (P < 0.01).

Fig. 5. Effect of theophylline on rate of fluid secretion induced by 5 x 10^-4 M DA (O) or 5 x 10^-4 M DA (●) on in vitro D. andersoni salivary glands. Control rate is the rate in DA alone. Number of salivary glands contributing to each mean is also indicated.

for many tissues (see Welt & Blythe, 1970). Calcium is involved in stimulus – secretion coupling in blowfly salivary glands (Prince & Berridge, 1973) and it may also function similarly in the salivary glands of ixodid ticks (J. R. Sauer, personal communication). Although Ca^{2+} concentrations in TC 199 and NRS are similar (Table 2), Ca^{2+} will be chelated by the high level of citrate in the latter medium, and this could possibly
Fluid secretion of ixodid ticks

Fig. 6. The effect of Tween 80 in NRS on rate of secretion of in vitro D. andersoni salivary glands. (A) when the stimulant drug is $10^{-4}$ M DA (○), and (B) when the stimulants are $10^{-3}$ M theophylline (▲) or $5 \times 10^{-3}$ M cyclic AMP (□). S.E. and number of glands contributing to each mean are indicated. A significant effect of Tween 80 is observed only when using $10^{-3}$ M theophylline.

Table 2. Comparison of the composition of human blood, TC 199 and NRS

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit of concentration</th>
<th>Whole blood*</th>
<th>Serum or*</th>
<th>TC 199</th>
<th>NRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>m-equiv/l</td>
<td>72–91</td>
<td>132–155</td>
<td>160</td>
<td>210</td>
</tr>
<tr>
<td>K⁺</td>
<td>m-equiv/l</td>
<td>39–62</td>
<td>3’6–4’8</td>
<td>8/4</td>
<td>8/6</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>m-equiv/l</td>
<td>3’0–3’7</td>
<td>1’7–1’9</td>
<td>0/8</td>
<td>1/3</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>m-equiv/l</td>
<td>4’8</td>
<td>4’3–6’1</td>
<td>2</td>
<td>1/8</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>m-equiv/l</td>
<td>71–87</td>
<td>93–110</td>
<td>125</td>
<td>131</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>mg/l</td>
<td>24–44</td>
<td>nil</td>
<td>30</td>
<td>nil</td>
</tr>
<tr>
<td>PO₄ (inorganic)</td>
<td>mg/l</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>Citrate</td>
<td>mg/l</td>
<td>nil</td>
<td>nil</td>
<td>550</td>
<td>nil</td>
</tr>
<tr>
<td>Malate</td>
<td>mg/l</td>
<td>nil</td>
<td>nil</td>
<td>2000</td>
<td>nil</td>
</tr>
<tr>
<td>Total lipid</td>
<td>g/l</td>
<td>4–7.2</td>
<td>3.8–6.8</td>
<td>nil</td>
<td>5</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>g/l</td>
<td>1.2–2.1</td>
<td>1.3–2.3</td>
<td>$3 \times 10^{-4}$</td>
<td>5</td>
</tr>
<tr>
<td>Tween 80</td>
<td>mg/l</td>
<td>380–530</td>
<td>754</td>
<td>3480</td>
<td>nil</td>
</tr>
<tr>
<td>Total amino acids</td>
<td>mg/l</td>
<td>18–25</td>
<td>8–54</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td>Glycine</td>
<td>mg/l</td>
<td>15–57</td>
<td>150</td>
<td>3350</td>
<td>nil</td>
</tr>
<tr>
<td>Proline</td>
<td>mg/l</td>
<td>40–106</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Glutamine</td>
<td>mg/l</td>
<td>0–13</td>
<td>150</td>
<td>8350</td>
<td>8350</td>
</tr>
</tbody>
</table>

* Approximate normal range of values for man (Spector, 1956).
Table 3. Effect of citrate on fluid secretion in isolated salivary glands of Amblyomma hebraeum

<table>
<thead>
<tr>
<th>Serial</th>
<th>TC 199 + 550 mg. l⁻¹</th>
<th>TC 199</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>190</td>
<td>340</td>
<td>79</td>
</tr>
<tr>
<td>2</td>
<td>123</td>
<td>258</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>320</td>
<td>554</td>
<td>73</td>
</tr>
<tr>
<td>4</td>
<td>115</td>
<td>425</td>
<td>270</td>
</tr>
<tr>
<td>5</td>
<td>266</td>
<td>409</td>
<td>54</td>
</tr>
<tr>
<td>6</td>
<td>298</td>
<td>370</td>
<td>24</td>
</tr>
</tbody>
</table>

\*P < 0.005 mean % increase 79

Pairs t-test; Simpson, Roe & Lewontin (1960).

account for the relatively low rate of fluid secretion in NRS. Accordingly, salivary glands of Amblyomma hebraeum were incubated in TC 199 supplemented with 550 mg. l⁻¹ citrate for 40 min, and maximum secretory rates were recorded following the addition of 10⁻⁶ M DA. The glands were then rinsed repeatedly with TC 199 lacking citrate and DA until salivation ceased. Maximum rates were recorded again following re-exposure to DA. The results are presented in Table 3; salivation rate was on average 80% higher following the removal of citrate.

A natural salivary stimulant

It has already been suggested that salivation is not controlled by a haemolymph-borne factor (Kaufman & Phillips, 1973b), but at least two objections to that experiment are that the specific ticks which donated the haemolymph may not have been salivating at the time and that a considerable time elapsed between collecting the haemolymph and applying it to the preparation (15–30 min). During that period all activity could have been lost if an inactivating enzyme had been present in the haemolymph. Consequently, I repeated the earlier experiments with Dermacentor haemolymph using the modifications specified in the Methods section. Fig. 7A shows that heat-treated haemolymph (taken from ticks which are salivating at the moment of exsanguination) does not stimulate secretion in test glands. These same glands were sensitive to DA whether or not the DA was heat-treated. Homogenates of the synganglion (= total CNS) were capable of stimulating secretion, and the threshold dosage was around 1 synganglion per 100 µl TC 199 (Fig. 7B). Secretory rate was 10–20% maximum at a dosage of 2 synganglia per 100 µl, which is equivalent to a DA concentration between 5 x 10⁻⁹ and 1 x 10⁻⁸ M. Homogenates of Malpighian tubule and ovary (in which the total wet weight of tissue was considerably higher than in the nervous tissue samples) were inactive.

Relation between weight of tick and rate of secretion

The feeding cycle of most female ixodid ticks can be divided into two distinct phases: a slow phase lasting 5–7 days (during which D. andersoni will have increased its weight from 10 to about 250 mg), and a rapid phase lasting 12–24 h (during which...
Fig. 7. Effect on in vitro secretion of *D. andersoni* salivary glands by (A) 1 part conspecific heat-treated haemolymph plus 2 parts TC 199 and (B) crude homogenates of conspecific synganglia in TC 199 at dosage indicated.

Fig. 8. Fluid secretory rates for salivary glands of *D. andersoni* excised at various phases of the feeding cycle. ●, Unfed; ○, partially fed; ▲, engorged. Vertical lines denote S.E. of the means, and the number of glands contributing to each mean is indicated. Differences between the means are significant (*P* < 0.05) up to about 100 mg tick weight. Glands from engorged specimens secrete at a significantly lower rate (*P* < 0.05) than those from partially fed ticks weighing between 100 and 250 mg. Stimulant is 10⁻⁴ M DA.
Fig. 9. Relation between female tick size (Amblyomma hebraeum) and weight of salivary gland pairs: O, wet weight; •, dry weight. The abscissa is in logarithmic scale. The relationship between time on the host and tick weight can be approximated as follows: the female took from 12 to 20 days to become fully gorged of which all but 1 or 2 days were required to reach about 500 mg. This somewhat long duration is possibly related to the fact that the adults of this species do not normally parasitize rabbits; thus the ticks (especially the females) were reluctant to attach, and even after having done so, were slow to commence feeding. Thereafter, however, they did feed normally reaching a replete weight from 1 to 3 g. Although A. hebraeum is much larger than D. andersoni (replete weight ranging between 0.5 to 1.2 g), the replete to unfed weight ratios are similar, as is the general pattern of feeding.

D. andersoni will have attained a replete weight of about 750 mg. Fig. 8 shows the maximum rate of secretion (10^-6 M DA) of glands dissected from ticks at various stages of the feeding cycle. Glands from unfed ticks can secrete in vitro, but their maximum rates are very low (4.0 ± 0.7 nl/min, n = 9). Slightly fed ticks (under 30 mg) show a marked increase in secretory rate (48 ± 8 nl/min, n = 19) and the increase in secretory rate is linear thereafter up to a tick weight of about 90 mg (Y = 2.2X + 8). Thereafter secretory rate remains at a constant level (217 ± 12 nl/min, n = 52) up to at least 250 mg. Glands from engorged ticks (570 ± 82 mg, n = 5) secreted considerably more slowly than did those taken from ticks in the latter part of the slow phase of engorgement. In this study most of the experimental D. andersoni ticks weighed 90-200 mg. In some cases, however, only smaller specimens were available (40-90 mg), so where a direct comparison with heavier ticks was necessary, a correcting factor, based on the linear portion of the curve in Fig. 8, was applied to the rates of the glands from smaller ticks.
Fluid secretion of ixodid ticks

Salivary glands from feeding male *D. andersoni* were prepared in the usual way and treated with DA. The threshold concentration of DA was higher for males (between 1 to $5 \times 10^{-8}$ M) than for females (about $2 \times 10^{-9}$ M). The maximum rate of secretion was $10 \pm 12$ nl/min, $n = 8$, or less than one-twentieth the maximum rate observed in feeding females weighing over 90 mg.

Wet and dry weights of salivary glands throughout the feeding cycle

Fig. 9 shows that although the wet weight of the salivary gland increases remarkably while the female tick is on the host, the dry weight increase does not parallel this curve. Male salivary glands (Fig. 10) show no significant increase in wet or dry weight throughout the feeding cycle.

Fluid secretion by other species of ixodid ticks

Salivary glands from feeding females of the following species were set up in vitro, treated first with $10^{-3}$ M ACh and followed by a series of DA concentrations after removing the ACh: *Dermacentor reticulatus*, *Boophilus microplus* Canestrini, *Rhipicephalus appendiculatus* Neumann, *Ixodes ricinus*, *I. hexagonus*, and *Hyalomma excavatum* Koch. In all cases secretion was triggered by low concentrations of DA (Fig. 11) but not by ACh. One may suggest from this uniform response that control of salivation is probably similar throughout the Ixodidae.
DISCUSSION

The characteristics of fluid secretion in TC 199 and in NRS differ in a number of respects. In TC 199 the maximum rate of secretion is higher, the threshold concentration at least for ADR is lower, and the response to adding a stimulant is quicker. Finally, whereas glands are insensitive to externally applied cyclic AMP in NRS, they do respond when bathed in TC 199. Although I have not conducted exhaustive tests to determine which differences in the composition of the two media are responsible for the generally better performance in TC 199, some speculation is possible. At least one trace constituent of TC 199 (Tween 80) can considerably modify the sensitivity of the salivary gland to certain stimulants of fluid secretion (Fig. 6). The mode of action of Tween 80 in this regard has not been adequately tested. Its action is probably not explainable solely in terms of altering the permeability of the cell, for although in NRS it potentiated the action of theophylline (Fig. 6), it had no effect on that of cyclic AMP. Also, theophylline had no stimulating effect on its own in TC 199 although Tween 80 is present in that medium. In any case, methyl xanthines generally can penetrate the cell membrane even though the latter is relatively impermeable to cyclic AMP (Robison, Butcher & Sutherland, 1971). Moreover, Tween 80 in NRS did not increase the response of salivary glands to high concentrations of DA (Fig. 6). Comparing this with the observation that when glands were transferred to TC 199 from NRS the rate of secretion increased by about 80% (p. 729), we must conclude that the difference between TC 199 and NRS in supporting salivary gland secretion cannot be explained on the basis of Tween 80 alone.

It is not surprising that tick tissues perform well in a culture medium designed for mammalian cells (i.e. TC 199). Indeed, salivary glands still secrete well after 7 days...
Fluid secretion of ixodid ticks

in culture in TC 199 supplemented with calf serum, but not after culture in NRS plus calf serum (Kaufman & Barnett, in preparation). In addition, one would expect an osmoregulatory organ to secrete most rapidly in a medium similar in composition to the fluid that it is normally called upon to secrete. NRS contains some substances in concentrations well above those characteristic for mammalian serum (see Table 2) – levels which could conceivably have adverse influence on secretion. In this regard, I considered that the high citrate level in NRS might have inhibited secretion by chelating Ca^{2+} (p. 732). That citrate does inhibit salivation when added to TC 199 is clear from Table 3. The inhibitory effect is probably not merely an osmotic one, since increasing the osmotic pressure by a similar amount using sucrose, NaCl, KCl or NaNO_3 affected salivation in isolated *Dermacentor* salivary glands only slightly, if at all (Kaufman & Phillips, 1973c). Further experiments would be required, however, before claiming with certainty that the principle mechanism of inhibition by citrate in the present experiment is by chelating Ca^{2+}.

The synganglion (comprising the total central nervous system) is a compact mass in the cephalothorax of ticks, and there is within the synganglion a factor(s) which can stimulate *in vitro* fluid secretion (Fig. 7B). Homogenates of ovary, Malpighian tubule and haemolymph from actively salivating ticks do not contain this factor, or if they do, it is apparently in insufficient quantity to be detected by bioassay. The localization of a stimulatory factor primarily in the CNS suggests that it may represent the neurotransmitter substance. At least three putative neurotransmitter substances have now been positively identified in the synganglion of *Boophilus* – ACh (Smallman & Schuntner, 1972), DA and NA (Megaw & Robertson, 1974). The factor which stimulates *in vitro* secretion cannot be ACh, but it could very likely be DA and NA. Now that there is some good morphological evidence that tick salivary glands are innervated (Coons & Rosshy, 1973; F. D. Obenchain, personal communication) it can be restated with some confidence that salivation in ixodid ticks is controlled neurally rather than hormonally. Although cholinomimetics are unable to stimulate *in vitro* secretion in at least 7 members of the ixodid family, they have been used as *in vivo* salivary stimulants for some years now (Howell, 1966; Tatchell, 1967a; Purnell, Branagan & Radley, 1969; Binnington & Schotz, 1973). Also, insecticides which block the action of acetylcholinesterase are able to stimulate salivation *in vivo* (Megaw, 1974). These results suggest that salivation is normally initiated by the firing of cholinergic nerves which in turn synapse with adrenergic secreto-motor nerves (Megaw, 1974). Such a scheme is somewhat analogous to that in the vertebrate autonomic nervous system in which postganglionic sympathetic (adrenergic) nerves are stimulated by preganglionic cholinergic fibres.

Fig. 8 shows that although the salivary glands of unfed ticks are capable of secreting fluid *in vitro*, they do so only at a very slow pace. As feeding progresses, however, the maximum secretory rate augments until a plateau is reached. The rising portion of the curve in Fig. 8 is composed of two segments: one, not including the unfed specimens, in which the weight of the female and the rate of fluid secretion are almost directly proportional, and the other, joining the unfed specimens to the slightly fed ones, in which the slope is far steeper.

The generally rising sector of Fig. 8 could possibly result from the *de novo* synthesis of secretory tissue during the slow phase of engorgement, since many workers have
noticed (see Balashov, 1965) that salivary glands of female ixodid ticks expand considerably in size during the same period. It seems from Fig. 9, however, that this marked expansion in salivary gland size is due almost entirely to an increase in water content alone rather than to any parallel increase in dry weight. Thus, unless the general pattern for Amblyomma in Fig. 9 does not hold for Dermacentor as well, some other explanation must be sought for the sensitization of the salivary gland during feeding. Moreover, it is still not clear what causes the very steeply rising portion of Fig. 8 (a 12-fold increase in secretory rate for a 60% increase in body weight). It is probable that a number of changes take place within the secretory cell during feeding and enable the secretory mechanism to operate more efficiently; for example, enzyme systems may be developing. It is also possible that in unfed specimens the receptors for the stimulant drug are not formed or for some other reason do not respond to the drug. Further experiments are underway to investigate this phenomenon. It is perhaps relevant to point out that a somewhat similar phenomenon has been observed in the blowfly, Calliphora. Although normally very sensitive to 5-hydroxytryptamine (5-HT) (Berridge & Patel, 1968), isolated salivary glands from freshly emerged adults do not secrete in the presence of 5-HT. Responsiveness to the hormone becomes apparent 2 h after emergence, although during this time, growth of salivary tissue is negligible (M. J. Berridge, personal communication). In the case of Calliphora, there is some suggestion that the canaliculi, which are believed to represent the pathway for fluid transport across the epithelium, are not fully developed in the freshly emerged adult. Alternatively, perhaps the receptors for the hormone are not fully differentiated, or for some other reason, events subsequent to receptor activation are not set in motion.

The dramatic reduction in secretory rate in glands from engorged ticks (Fig. 8) is not unexpected. As the female feeds only once as an adult, most of the tissues are resorbed after the meal - even the endocuticle which was synthesized only a matter of days earlier (Lees, 1952). Histological disorganization of salivary tissue following engorgement has frequently been reported (Till, 1961; Balashov, 1965), and this phenomenon could be responsible for the loss of function observed in Fig. 8.

Male ticks engorge only modestly as adults, barely doubling the unfed weight in the case of D. andersoni. The male of Ixodes ricinus usually takes no blood meal (Balashov, 1965). Except for the occasional report (Gregson, 1943), only female ticks are known to transmit tick paralysis (Gregson, 1962). These observations suggest that males secrete very little saliva in comparison with females and that it is probably only the female gland which functions as an osmoregulatory organ. It is consistent with this opinion that male salivary glands secrete in vitro at only a small fraction of the rate of female glands (page 737) and that there is no change in wet or dry weight with feeding (Fig. 10). These results may have a bearing on the identity of the fluid secretory cell in the salivary gland of ixodid ticks. Although nobody has yet conclusively established which cell(s) is mainly concerned with fluid transport in tick salivary glands, there is ultrastructural evidence that the so-called ‘water cell’ in the fundus of the Group III acinus is implicated (Meredith & Kaufman, 1973). It has also been suggested that the Group I acinus may be involved (Kirkland, 1971; Balashov, 1972; Coons & Rosdy, 1973). The present evidence that male glands secrete only feebly in vitro, even though both sexes are endowed with Group I acini, detracts somewhat from the second
hypothesis. *Ornithodoros moubata*, an argasid tick which apparently secretes little, if any, saliva during feeding (Kaufman, 1971), also possesses prominent Group I acini, the structure of which is very similar to that of ixodid ticks (P. A. Diehl, personal communication).

When female glands of *D. andersoni* are exposed to DA, the group III acini swell up (unpublished observations) - presumably an indication that their lumina are filled with secreted fluid. Group I acini do not show this visible effect in the presence of DA. Till (1961), Chinery (1965) and Balashov (1965, 1972) all report that histological changes of Group I acini are virtually negligible throughout the feeding period of both sexes. From the light microscopic observations of Till (1961), it seems that the type 'e' cell of *Rhipicephalus appendiculatus* may be homologous to the water cell of *D. andersoni*. Till points out that in males, the type 'e' cells remain inconspicuous throughout the feeding period whereas they undergo considerable change in females. Recently, cells homologous to the water cell of *D. andersoni* have been detected in *B. microplus* (M. J. W. Megaw, personal communication) and in *Amblyomma hebraeum* (P.A. Diehl, personal communication). In both these ticks the water cells become easily recognizable as such only once feeding is underway. At least in the case of *Boophilus*, changes comparable to those observed for adult females seem not to occur in larvae, nymphs or adult males throughout the feeding period. This may explain why both Coons & Roshdy (1973) and Kirkland (1971) failed to notice typical water cells, since Kirkland dealt with nymphal glands, and Coons & Roshdy with the glands of unfed males.

Establishment of the *D. andersoni* colony was made possible through generous gifts of specimens from Mr P. R. Wilkinson, presently of the Canada Department of Agriculture Research Station at Lethbridge, Alberta. Miss Brenda Vidler of Fisons Ltd provided specimens of *B. microplus* and *R. appendiculatus*, and Dr Rolph Immler of Ciba Geigy Ltd provided specimens of *A. hebraeum*. Pye-Unicam Ltd, Cambridge, graciously allowed me the use of a flame spectrophotometer.

I am particularly grateful to Dr M. J. Berridge for his most valuable comments on the MS and to Dr S. F. Barnett for his continual enthusiasm and encouragement. Most of this work was completed in his Cambridge laboratory and was made possible by the Wellcome Trust's generous financial support including a post-doctoral veterinary research fellowship to the author. That work done in Switzerland was generously supported by the Fonds National Suisse for scientific research (Request No. 3.3460.74).

Finally, I wish to acknowledge the editor and referee for their valuable suggestions - particularly for that concerning the possible effect of citrate.

**REFERENCES**


