antenna is amply adequate to guide the unilaterally antennectomized insects to the food choices, as evident by very minor and negligible numerical differences in maiden biting response between unilaterally antennectomized and normal individuals.

It may be seen from the extremely poor biting response by the bilaterally antennectomized beetles, in comparison to normal or unilaterally antennectomized ones, that the antennal chemoreceptors perform as main olfactory sensilla in the insects' food-plant finding, while the maxillary and labial chemoreceptors, if at all present, exhibit only a secondary role. Besides, the antennal chemoreceptors seem to be chiefly associated with the attraction of the pest towards its food plant and not with the 'avoidance' as suggested by Thorsteinson[3] based on Chin's[4] work on the Colorado potato beetle, *Leptinotarsa decimlineata* (say) (Coleoptera: Chrysomellidae).

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The relationship of phosphodiesterase and cyclic AMP to the process of fluid secretion in the salivary glands of the ixodid tick *Amblyomma americanum*[1]

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Summary. Phosphodiesterase (PDE) activity in the salivary glands of the female. *Amblyomma americanum* decreased as the tick progressed from a slow to a rapid phase of feeding, while the rate of fluid secretion increased when glands were stimulated with cyclic AMP and theophylline. Dopamine stimulated PDE activity and an 'inhibitory' factor was found in glands obtained from rapidly engorging ticks which decreased PDE activity. These findings are discussed as they relate to the process of fluid secretion by salivary glands of feeding ixodid ticks.

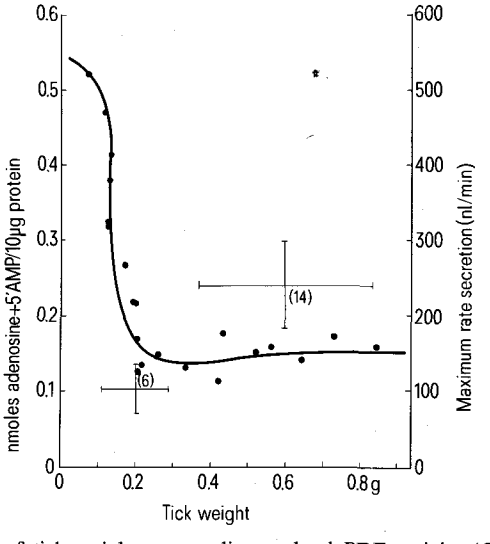
Female ixodid ticks must have effective osmoregulatory systems because of their ability to concentrate large blood meals, and are excellent animals in which to study the problem of ion and water balance.

In the process of concentrating its blood meal, excess fluid moves across the gut epithelium and is expelled via the salivary glands back into the host[2,3]. We have recently obtained evidence that implicates both cyclic AMP (cAMP) and Ca^{+2} in the process of salivary gland fluid secretion in females of the lone star tick *Amblyomma americanum*[4–10]. Kaufman[11] and Sauer et. al,[10] have demonstrated that if salivary glands of female ixodid ticks are stimulated in vitro with catecholamines, the rate of fluid secretion is highest in glands obtained from rapidly engorging ticks. Catecholamines may stimulate fluid secretion by causing an increase in the steady state level of cAMP which acts as an intracellular second messenger mediating the signal of the primary transmitter[12]. Recently, we reported that cAMP mimics the stimulatory effect of catecholamines[5,6]. Furthermore, changes in levels or activity of cAMP, adenylyl cyclase and/or phosphodiesterase (PDE) may change as states of engorgement change. In this preliminary communication, we report the relationship of phosphodiesterase and cAMP to the process of fluid secretion by salivary glands of the female lone star tick.

Materials and methods. Female *A. americanum* in 2 phases of feeding were used; a slow phase lasting 6–12 days (weight increased from ~4 mg to ~300 mg) and a rapid phase lasting 12–24 h (weight increased from ~300 mg to ~800 mg). Pairs of salivary glands were dissected out in 50 mM Tris-HCl buffer containing 5 mM $MgCl_2$ adjusted to pH 8. After dissection, glands were placed in small glass homogenizers containing 250 µl of Tris buffer and homogenized. Some homogenates of salivary glands from the 2 feeding phases were pooled prior to assay.

Additional tick salivary gland pairs were dissected in a Ringer solution with MOPS buffer to retain maximal secretory ability[8]. One gland was bathed in Ringer solution and prestimulated with 10^{-6} M dopamine for 5 min, rinsed with Tris buffer and homogenized as before. The other gland was incubated in the same solution but without dopamine and served as the control.

PDE activity was measured by taking 50 µl of salivary gland homogenate added to ½ dram glass vials containing 6 nmoles cAMP in 10 µl of Tris buffer and trace amounts of H^3 cAMP. Reactions were allowed to proceed for 10 min at



Effect of tick weight upon salivary gland PDE activity (●) and maximal secretion rate by in vitro salivary glands stimulated with cyclic AMP (10^{-2} M) and theophylline (10^{-2} M). Horizontal lines represent weight range of ticks from which glands were obtained (0.105–0.289 g in slowly feeding ticks and 0.364–0.825 g in rapidly feeding ticks). Vertical lines represent ±SD of the maximum rate of secretion. Numerals in parenthesis indicate numbers of experiments.

37 °C and then terminated by addition of 10 µl 2M HCl. Phosphodiesterase activity of homogenates was measured using the paper chromatographic method of Bielinska and Piechowska[13].

The technique of Needham and Sauer was used to measure the effect of 10^{-2}M cAMP and 10^{-2}M theophylline on in vitro salivary fluid secretion by glands obtained from ticks in various stages of engorgement using a support medium of modified TC-199[10,11]. Statistical analysis was performed using Student's t-test. All data are expressed ±SD.

Results. The figure shows the effect of tick weight upon salivary gland PDE activity. Activity drops sharply in glands obtained from ticks in slow phase of engorgement and reaches a steady-state level in glands obtained from ticks in the rapid phase of feeding. The figure also shows that cAMP and theophylline stimulated secretion more in rapidly feeding ticks (average maximum 240 nl/min) than in slow feeding ticks (average maximum 105 nl/min). These differences are significant at $p < 0.001$ level.

When salivary gland homogenates from ticks, either in a rapid or slow phase of feeding were pooled (table 1), the combined PDE activity was near that observed in glands from ticks in a rapid phase of feeding. Prestimulation of glands with 10^{-6} M dopamine (table 2) caused in all cases higher PDE activity.

Discussion. These experiments establish that PDE activity drops sharply (~70%) just prior to the tick entering rapid engorgement and thereafter, remains at low levels. These results correlate well with the observation that the secretory rates of salivary glands increase greatly during the same period when stimulated by cAMP and theophylline. The same type of secretory response is noted when salivary glands are stimulated by catecholamines[6,7]. These findings lend credence to the hypothesis that cAMP plays an important role during fluid secretion. The high PDE activity in glands obtained from the lighter ticks may be one reason why the secretory rate is correspondingly low. The PDE activity in lighter ticks may be at such high steady state levels that even if maximal stimulation of glands by catecholamines occurred, the induced cAMP would be destroyed before it is able to substantially affect fluid secretion.

Table 1. PDE activity in pooled homogenates of salivary glands from ticks in 2 phases of feeding

Tick weight	5′−AMP+ADE (nmoles)/10 µg protein
>0.300 g (rapid phase of feeding)	0.19±0.07 (3)*
<0.300 g (slow phase of feeding)	0.56±0.13 (3)
Pooled homogenate	0.17±0.09 (3)

PDE activity in glands from ticks <0.300 g varied significantly from PDE activity in glands from ticks >0.300 g and pooled homogenates ($p < 0.05$; t-test). * Numerals in parenthesis indicate numbers of experiments.

Table 2. PDE activity in salivary glands preincubated in a Ringer solution with or wirhout 10^{-6} M dopamine.

Tick weight	Activity in gland pairs 5′−AMP+ADE (nmoles)/10 µg protein Control	Prestimulated gland
0.140 g	0.41	0.62
0.204 g	0.44	0.67
0.210 g	0.35	0.60
0.226 g	0.16	0.23
0.263 g	0.28	0.29
0.320 g	0.28	0.40
0.520 g	0.16	0.36

Control and prestimulated gland PDE activity are significantly different ($p < 0.05$; t-test).

The deactivation of PDE which occurs in the salivary glands of rapidly engorging tick (figure) and the results of the pooling experiments (table 1) suggest that an inhibitor of PDE may be present in glands from rapidly feeding ticks. It is worth noting that Wang and Desai[14] have reported a protein in bovine brain that acts as a competitive inhibitor of the Ca^{+2} activated activator of PDE.

The fact that both PDE activity and fluid secretion increase when tick salivary glands are stimulated by dopamine suggests an effective feedback mechanism for control of secretion. Recently Needham and Sauer[8] suggested that cytoplasmic levels of Ca^{+2} may rise in salivary glands after stimulating glands of *A. americanum* with dopamine. An increased cytoplasmic level of Ca^{+2} may help account for the rise in PDE activity seen when glands are stimulated by dopamine by activating a Ca^{+2} dependent protein activator of PDE, a factor often associated with PDE[15]. Also Revuelta et al.[16] have shown that when dopamine stimulates formation of cAMP the newly formed cAMP activates PDE by causing release of a membrane-bound protein activator of PDE in rat brain tissue. The increased PDE then returns cAMP to basal levels.

Kaufman et al.[17] have demonstrated that Na^+, K^+-ATPase levels in *A. habraeum* salivary glands rise significantly during the rapid phase of feeding which correlates with increased secretory rates seen at the same time. Na^+, K^+-ATPase in many cases are phosphorylated before activation and phosphorylation of some component of a cell is the only known mechanism through which cAMP is known to regulate cellular activity[18]. Thus it seems possible that fluid secretion, cAMP, and the Na^+, K^+-ATPase 'pump' are all closely related.

In conclusion, the results of the present experiments show an inverse relationship between tick salivary gland PDE activity and gland fluid secretory capability. Pooled enzyme experiments suggest the presence of an inhibitor of PDE in glands from rapidly engorging ticks, and results indicate that a primary effector of fluid secretion (dopamine) also affects PDE activity.

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