

# Regulation of body volume by salivation in a tick challenged with fluid loads

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KAUFMAN, W. R., A. A. AESCHLIMANN, AND P. A. DIEHL. *Regulation of body volume by salivation in a tick challenged with fluid loads.* Am. J. Physiol. 238 (Regulatory Integrative Comp. Physiol. 7): R102-R112, 1980.—Injection into the hemolymph of 1.2% NaCl, 11.2% sucrose, 2.3% urea (all approximately isosmotic to hemolymph), or distilled water induced salivary fluid secretion in the ixodid tick *Amblyomma hebraeum* Koch. Saline gave the largest response at high doses. Injection of hyperosmotic NaCl into the hemolymph did not induce salivation but led to the drinking of distilled water in amounts sufficient to dilute the salt load to isosmolality. Atropine only partially inhibited salivation induced by NaCl, sucrose, and distilled water. Reserpine markedly inhibited salivation induced by NaCl. We propose that at least two sensory pathways (one cholinergic, one not) converge on the secretory nerve. The physiological significance of the cholinergic pathway is not known. The other pathway probably mediates the regulation of hemolymph volume, possibly via stretch receptors, but its transmitter is not known.

ixodid tick; *Amblyomma hebraeum* Koch; salivary fluid secretion; atropine; reserpine; hemolymph volume regulation

A FEMALE IXODID TICK (*Acar*i, Ixodoidea) can increase its net weight almost 100-fold over the 7- to 10-day duration spent in sucking blood from a mammalian host. It excretes the excess fluid imbibed with the blood meal back into the host via the salivary secretions, and thus concentrates the protein (26). A recent series of papers has indicated that the nerves controlling fluid secretion are catecholaminergic (5, 6) and that a cholinergic element impinges either directly or indirectly on the secretory nerves (7). Although it was reasonable to postulate that the cholinergic nerve is situated in some part of the sensory or integrative pathway, one still has only a vague notion about what sensory information might be monitored for the purpose of triggering salivation.

For example, Kaufman and Phillips (8) observed that the hemolymph compartment in the ixodid tick *Derma-centor andersoni* remained a constant proportion of body weight over the whole feeding cycle despite the enormous volume of fluid passing through the hemolymph (a volume estimated at around 150–200 times the unfed weight of the tick!). At that time we proposed that volume regulation of the extracellular space was accomplished by the salivary glands, as neither transpiration of water through the integument nor the volume of the fecal-urine mixture was sufficient to account for water balance dur-

ing feeding. Also, ticks maintain a hemolymph concentration hyperosmotic to the imbibed meal, and natural saliva is slightly hyposmotic to the hemolymph (8). It thus seems possible that the salivary gland plays a role in osmoregulation as well, although the extent of the tick's power to achieve osmoregulation when fed artificial meals is a question remaining open to investigation. The present study was undertaken in *Amblyomma hebraeum* Koch to determine whether the tick salivary gland is able to regulate hemolymph volume when challenged with various loads injected directly into the hemolymph.

## MATERIALS AND METHODS

Adult *A. hebraeum* ticks were obtained from laboratory stocks of Ciba-Geigy AG, Station Les Barges, Vouvry (VS), Switzerland. Males and females (at least 3 mo old) were fed on rabbits. For most experiments, females were removed from the host when they reached a weight of 150–600 mg. All solutions were injected as previously described (7). Briefly, a 30-gauge needle attached to an Agla micrometer syringe (Wellcome Reagents) containing the desired fluid was introduced through the soft membrane at the capitular-scutal articulation. About 2 min after injection the ticks were removed from the microscope stage and were kept on a special observation table where saliva was collected in calibrated glass capillary tubes for up to 5 h if necessary. Volume of secretion is expressed as a percent of the injected volume. Osmotic pressure of hemolymph and saliva was determined with a Wescor 5100B vapor pressure osmometer.

Atropine sulfate and dopamine HCl were purchased from Sigma Chemicals, and reserpine phosphate was a gift from Ciba-Geigy AG, Basel. Other chemicals were of reagent grade from various sources.

*Measurement of hemolymph volume.* [Carboxyl-<sup>14</sup>C]-inulin, 24.5 mg (50  $\mu$ Ci) in 10 ml sterile water, was purchased from New England Nuclear. This solution, normally 1  $\mu$ l (5 nCi)/100 mg body wt, was injected directly into ticks. When an injection volume greater than 1  $\mu$ l/100 mg was desired, the inulin solution was diluted to maintain the tracer level at 5 nCi/100 mg body wt.

[1,2-<sup>14</sup>C]polyethylene glycol (4,000 mol wt; 250  $\mu$ Ci in 454 mg) was supplied by New England Nuclear. Prior to injection as a hemolymph space marker it was dissolved in vehicle (1.2% NaCl, distilled water (DW), or 11.2% sucrose, as appropriate) so that the total dose was 6.5

nCi/100 mg body wt regardless of the vehicle volume.

After the marker had equilibrated in the extracellular space (approx 1 h, see Fig. 7), the dorsal abdominal cuticle was slit with a razor blade scalpel; the exuding hemolymph was collected into calibrated microcapillary tubes (1–10  $\mu$ l) and was transferred directly to scintillation vials containing 10 ml Bray's solution (3).  $^{14}$ C activity (corrected for background) was determined on a Nuclear-Chicago Mk II scintillation counter at 70% counting efficiency. Hemolymph volume (i.e., inulin space or polyethylene glycol (PEG) space) was calculated from the formula:  $(V_i \times \text{cpm}_i \times V_s) \div \text{cpm}_h$ , where  $V_i$  is the microliters of fluid injected,  $\text{cpm}_i$  the counts per minute per microliter of injection medium,  $V_s$  the microliters of hemolymph sampled, and  $\text{cpm}_h$  the counts per minute of the hemolymph sample. Since over the time course of the experiments there was no detectable excretion of either inulin or PEG, no correction for excretion was necessary.

Both inulin and PEG were excluded from the saliva and did not appear in the urine at least within the first 24 h after injection. Nor was there any significant uptake of either substance by Malpighian tubules, salivary glands, ovary, or gut as determined from radioactivity associated with homogenates of these tissues. Both inulin and PEG, being excluded from cells, being neither metabolized nor excreted to any measurable extent during the course of these experiments, appear to be suitable markers for the extracellular space, which in arthropods is normally equated with the hemolymph.

## RESULTS

Ticks were injected with varying loads of 1.2% NaCl, 11.2% sucrose, or 2.3% urea (all wt/vol), and the ensuing course of salivation was recorded. These concentrations were originally chosen because they are isosmotic with the hemolymph of partially gorged *D. andersoni* ( $375 \pm 6$  mosmol/l; mean  $\pm$  SE,  $n = 22$ ) (8). Because the hemolymph of *A. hebraeum* turns out to be only slightly less concentrated ( $360 \pm 11$  mosmol/l; mean  $\pm$  SE,  $n = 15$ ) than that of *D. andersoni*, the injected fluids were on average no more than 5–6% hyperosmotic to the hemolymph. Figure 1A shows that with 1.2% NaCl salivation increases with increasing load. Concomitantly the average latency falls until a minimum is apparent at 25  $\mu$ l/100 mg body wt (Table 1). Figure 2 illustrates the time course of salivation in ticks loaded with 25  $\mu$ l 1.2% NaCl/100 mg. On the average, secretion began within 5 min and had virtually ceased within 1 h. When these ticks were rechallenged with the same load, the second response was very similar to the first (Fig. 2).

Ticks responded to the remaining substances (glucose, sucrose, urea, DW) in a manner by and large similar to NaCl up to a dose of 10  $\mu$ l/100 mg body wt. In all cases, however, 25  $\mu$ l/100 mg were inhibitory when compared to the response to saline (Fig. 1). The latency to salivation tended to be an inverse function of the secretory response. Thus for saline at 25  $\mu$ l/100 mg, a vigorous response was accompanied by a short latency (Table 1). With the other substances, for which there was no clear increase in response with increased dose (Fig. 1A), laten-

cies to salivation were also dose independent (Table 1). Furthermore, the latency following injection of 5 or 10  $\mu$ l saline/100 mg was very similar to the latency for other fluids that elicited responses of similar magnitude. Finally, as will be shown in more detail below, as the secretory response to a given dose of saline diminishes with time following removal from the host, so does the latency increase (Fig. 6). If the salivary glands do indeed constitute an osmoregulatory system it would be more informative to express their activity in terms of severity of the challenge. Thus in Fig. 1B we show salivary secretion as a percentage of the injected volume. The discrepancy between saline and the other fluids is still clearly apparent at 25  $\mu$ l/100 mg body wt.

We next tested whether one could elicit salivation by raising the osmotic concentration of the hemolymph. Ticks were injected with 2  $\mu$ l/100 mg body wt of 2 M NaCl. With the assumption that the salt is retained to a large extent in the extracellular space, these injections should have raised the osmolality of the hemolymph by about 40–45%. Total secretion for three ticks was 2.8, 0, and 0% of the injected volume. Since a previous study showed that isolated salivary glands of *D. andersoni* could not secrete a fluid hyperosmotic to the bathing medium (10), it seemed worth considering that ticks injected with hyperosmotic saline might attempt instead to regulate hemolymph concentration by drinking a hyposmotic fluid if given the chance. Accordingly, ticks were injected with 2 M NaCl (3  $\mu$ l/100 mg) and then capillary tubes partially filled with DW were placed over their mouthparts. Control ticks were injected with 3  $\mu$ l 1.2% NaCl/100 mg. Figure 3 shows that ticks injected with hyperosmotic saline imbibed, within 4.5 h, more than enough water to dilute the injected load to isosmolality. The controls, however, also drank a considerable volume (approximately half the amount as did the experimentals).

The foregoing series of experiments demonstrated that salivation could be provoked by injecting fluid loads and that by far the most effective stimulus was a large volume of 1.2% NaCl. Because all these challenges most likely provoked salivation by activating sensory receptors, it was important to see which stimuli could be attenuated by atropine, a drug known to abolish pilocarpine (PC)-induced salivation (7). Ticks were injected with 1.2% NaCl, 11.2% sucrose, or DW. Each of these fluids contained 1.45  $\mu$ mol atropine/ml, which resulted in a final dose to the ticks of 145  $\mu$ mol atropine/kg body wt. Atropine caused a significant reduction in NaCl- and DW-induced salivation (Fig. 4). Atropine also appeared to increase the latent periods (Table 1). Note, in most cases, the large standard errors of the mean, indicating much variation in response to all these injections (Figs. 1 and 4; Table 1).

Reserpine reduces the salivary response to PC (7), possibly by depleting the secretory nerves of their catecholamine transmitter (21). We thus tested whether reserpine would likewise inhibit salivation normally induced by 1.2% saline. Ticks were injected with 2  $\mu$ l/100 mg body wt of a fluid containing reserpine phosphate (7  $\mu$ mol/ml). This constituted a final dose of 140  $\mu$ mol/kg, a dose found previously to diminish PC-induced saliva-

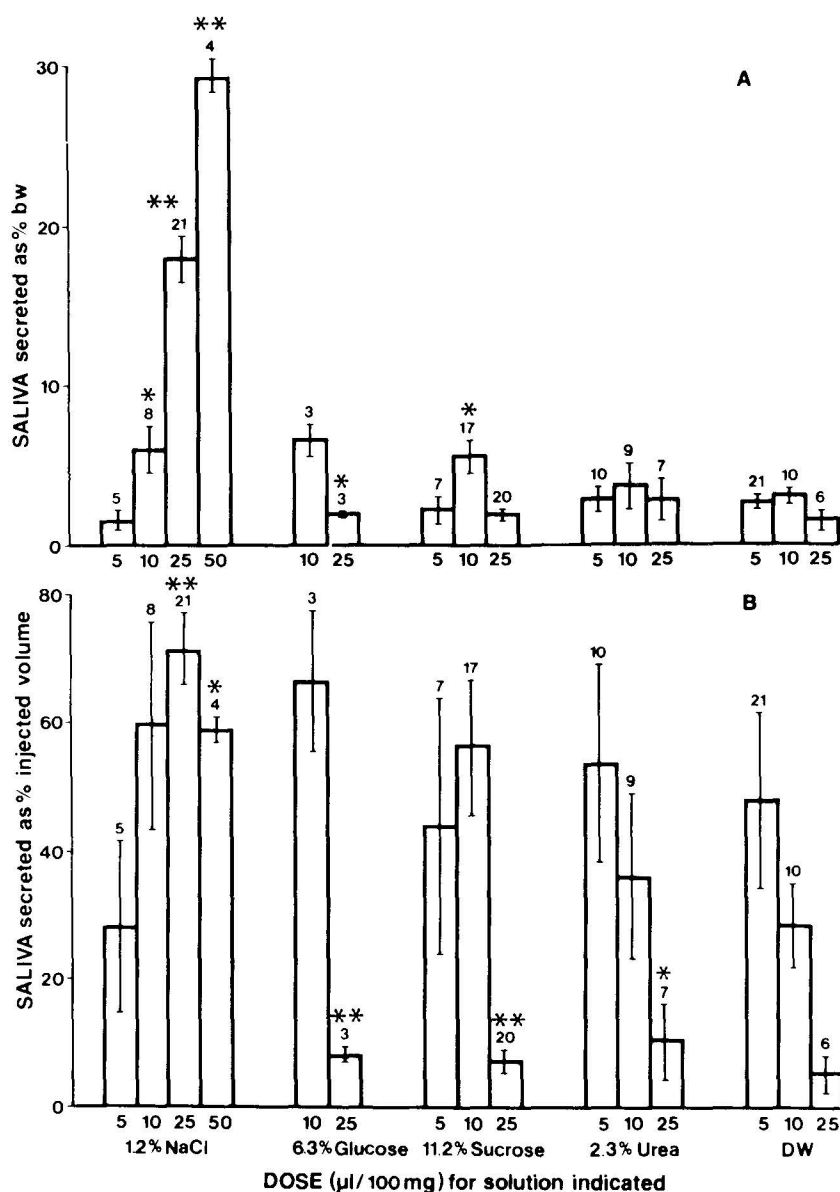


FIG. 1. Secretory response of ticks injected with various fluid loads as indicated. Standard errors and  $n$  are shown for each treatment. Asterisks denote statistical differences from the lowest dose in each case:  $*0.05 > P > 0.01$ ;  $**P < 0.01$ . In *A*, secretion is expressed as %body wt; in *B* it is expressed as %injected volume. Doses of 1 and 2  $\mu\text{l}/100\text{ mg}$  body wt were shown earlier (7) to elicit no secretion.

tion by 70% (7). Controls received 20 mg  $\text{Na}_2\text{HPO}_4/\text{kg}$ . Twenty-three hours later, the saline load (25  $\mu\text{l}/100\text{ mg}$  body wt) was injected. Both the reserpinized and control ticks responded only very weakly to this normally potent challenge, 11 of 12 ticks secreting less than 3% of the injected load after 70 min (Fig. 5A). All the ticks were subsequently injected with a further 25  $\mu\text{l}$  saline/100 mg body wt. Figure 5B displays the ensuing pattern of secretion. In the reserpinized ticks, the latency was considerably lengthened, the rate of secretion remained very low, and, in the end, less of the saline load was excreted compared to the controls.

Failure of the control ticks (Fig. 5A) to salivate after the usually potent stimulus of 25  $\mu\text{l}$  saline/100 mg suggested that response to the stimulus might be a function of the time intervening between removal of the tick from the host and injection of the fluid. Figure 6 shows that as early as 90–120 min postremoval, the salivary response to saline injection was reduced by almost 50%, and by 37 h the ticks were unresponsive to the load. We considered

the possibility that urinary excretion by the tick might have been partially responsible for the decline in salivation, in that some fraction of the injected load would have served merely to restore the body to its prediuretic volume (doses were calculated from the weight immediately prior to injection). However, since total weight loss by 48 h postremoval was only  $6.1 \pm 0.8\%$  (mean  $\pm$  SE,  $n = 34$ ) of the initial weight recorded, this factor could not have accounted for the decline in salivation. Moreover, as will be demonstrated below, hemolymph volume remains stable over this period, and the salivary glands are capable of responding to other stimuli (7). Clearly then, other changes must be responsible for the attenuated salivation response observed in Fig. 6 (see DISCUSSION).

In the case of all the treatments recorded in Fig. 1B, the ticks excreted only a fraction of the fluid injected. Thus our aim was to examine to what extent the observed salivary responses did in fact rectify hemolymph volume. Both [ $^{14}\text{C}$ ]inulin and [ $^{14}\text{C}$ ]polyethylene glycol were used to measure hemolymph volume by standard dilution

TABLE 1. Latencies to secretion under various experimental conditions

Fluid Injected	Dose, $\mu\text{l}/100$ mg body wt	Latency, min	
		Fluid alone, control	Fluid plus atropine, $145 \mu\text{mol}/\text{kg}$ body wt
1.2% NaCl	5	$46 \pm 12$ (3)	
	10	$25 \pm 10$ (6) *	$28 \pm 12$ (6)
	25‡	$4.4 \pm 0.8$ (44)*	$12 \pm 2$ (13)
	50	$23 \pm 19$ (4)	
11.2% Sucrose	5	$35 \pm 17$ (5)	
	10	$36 \pm 8$ (13)*	$92 \pm 17$ (6)
	25‡	$14 \pm 4$ (17)	
2.3% Urea	5	$31 \pm 10$ (7)	
	10	$24 \pm 9$ (6)*	$115 \pm 10$ (3)
	25‡	$17 \pm 5$ (6)	
DW	5	$39 \pm 7$ (15)	
	10	$46 \pm 12$ (10)†	$93 \pm 27$ (7)
	25‡	$38 \pm 28$ (4)	

Values are means  $\pm$  SE, with  $n$  given in parentheses. DW, distilled water. Values significantly different by Student's  $t$  test: \*  $P < 0.01$ ; †  $P < 0.05$ . ‡ At this dose latency for saline-induced secretion was significantly less than that for the other fluids.

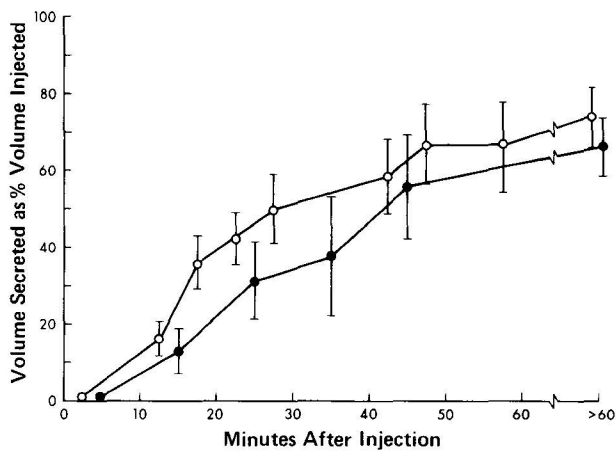


FIG. 2. Time course of secretory response to  $25 \mu\text{l}$  1.2% NaCl/100 mg body wt. These 5 ticks were each injected a second time with a volume of 1.2% NaCl equal to that secreted as a result of the initial injection. Standard errors are indicated. ○, First injection; ●, second injection. Response to second injection was not significantly different from that to first injection.

techniques (see METHODS). Figure 7 shows that although the tracer equilibrates in the hemolymph compartment by 15–30 min in some cases, the standard errors indicate a large variation. Between 1 and 5 h, estimates of hemolymph volume were far more consistent and were time independent; in subsequent experiments at least 1 h was allowed for uniform tracer distribution.

The PEG space was approximately 34% larger than the inulin space (Table 2). When expressed as a percentage of body weight, the hemolymph volume of engorged ticks is less than half that in partially fed ticks (Table 2). These values remain more-or-less unchanged within the 1st wk postremoval or postengorgement, although by 10–

14 days (when egg laying is underway) there is a suggestion of a slight recovery in hemolymph volume of the engorged ticks (Fig. 8).

Tables 3 and 4 show how injections of DW, saline, and sucrose perturb hemolymph volume. Of the  $5 \mu\text{l}$  DW/100 mg injected, an extremely variable proportion, averaging 60%, was secreted (Table 3). When administered 40–48 h postremoval the volume of saliva induced by the injection is significantly less than is the case for ticks receiving DW within 1 h of removal (Table 3). There is no significant difference, however, between hemolymph volume of the control ticks (A) and that of either of the two experimental groups (B and C). The fact that hemolymph volume is not elevated in those ticks that secreted less saliva indicates what one might have predicted, namely that the injected water is not confined to the extracellular space.

The situation is different for those ticks receiving 11.2% sucrose or 1.2% saline (Table 4). Since those receiving sucrose ( $10 \mu\text{l}/100$  mg) secreted on average 22% of the load, one would expect the hemolymph volume to rise from 23.4 to 31.2% body wt were the injected fluid retained in the extracellular space. The observed value of 31.1% conforms to this expectation. Similarly, because on average only 8% of the injected sucrose load was excreted when the dose was increased to  $25 \mu\text{l}/100$  mg, one anticipated a resultant hemolymph volume of 46.4% body wt. The observed value of 48.6% also conforms reasonably to expectation. Interestingly, although ticks receiving  $25 \mu\text{l}$  1.2% NaCl/100 mg 1 h postremoval secreted only 76% of the injected fluid, the resultant hemolymph space was not significantly elevated above the control value (Table 4). Hemolymph volume was elevated as expected when injection occurred 48-h postremoval (Table 4, treatment E), due to the attenuation in salivation response.

The close correlation between hemolymph volume and saliva volume is further emphasized in Fig. 9. After injection of a fluid load one would predict the final hemolymph volume to be an inverse linear function of the saliva volume, provided that salivation is the sole means for removing fluid from the hemolymph. The analysis presented in Fig. 9 leaves little doubt that such is the case for the near-isosmotic fluids.

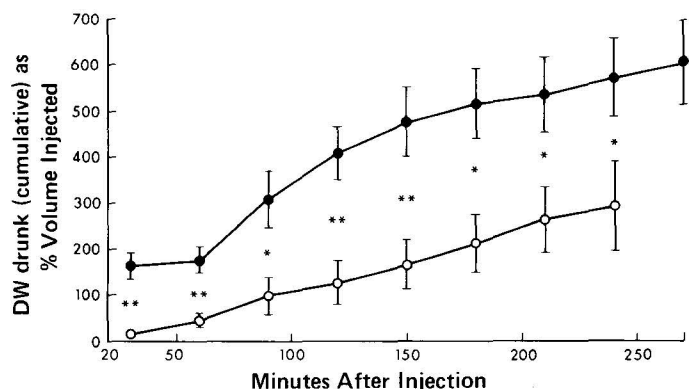


FIG. 3. Drinking response of ticks injected with hyperosmotic saline. Following injection, ticks were offered DW to drink from capillary tubes placed over their mouthparts. ●, Injected with 2 M NaCl,  $3 \mu\text{l}/100$  mg body wt ( $n = 8$ ); ○, control, injected with 1.2% saline,  $3 \mu\text{l}/100$  mg body wt ( $n = 6$ ). Standard errors are indicated. Significance: \* $0.05 > P > 0.01$ ; \*\* $P < 0.01$ .

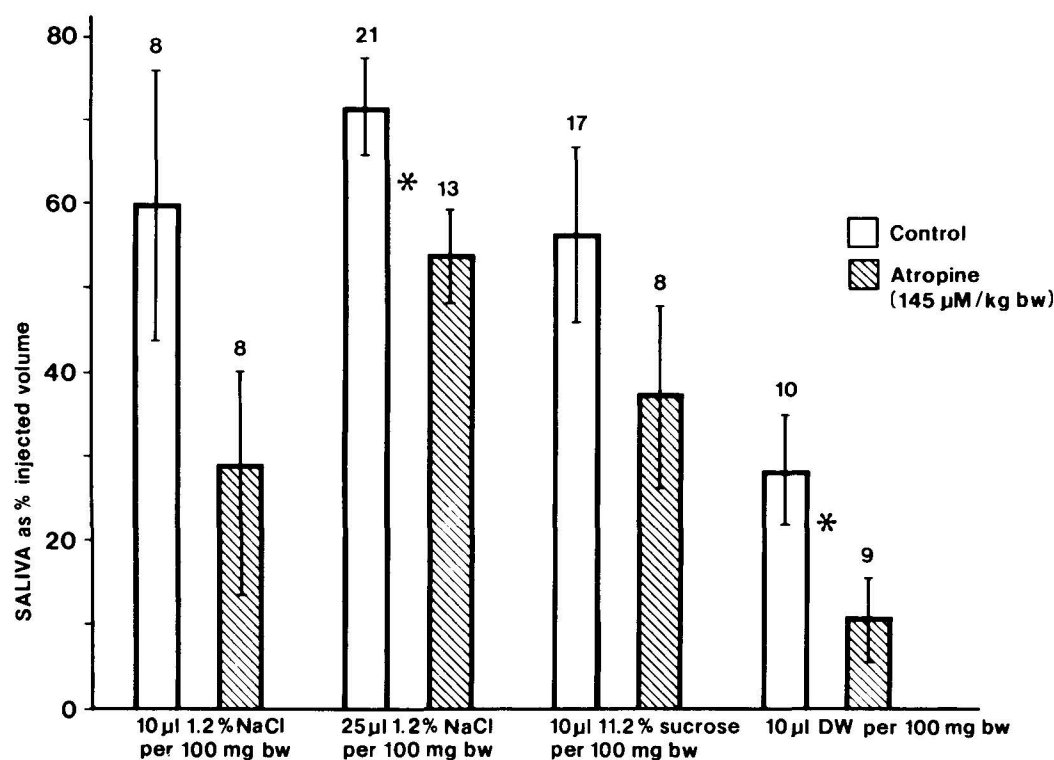


FIG. 4. Effect of atropine (145 µmol/kg body wt) on salivary secretion induced by various fluids (as indicated). Open bars, controls;

hatched bars, treated with atropine. Standard errors,  $n$ , and statistical significance are indicated.

The weak response to large volumes of sucrose (Table 4) is probably explained by inability of the tissue to secrete this fluid efficiently, as indicated by the following experiment. Ticks were administered 11.2% sucrose (25 µl/100 mg); as expected, the salivary response was weak, only  $3.9 \pm 1.4\%$  (mean  $\pm$  SE,  $n = 7$ ) of the injected load being secreted in 80 min. The ticks were reweighed and then injected with a solution of dopamine (DA) in 11.2% sucrose, the dose being 53 nM DA in 1 µl/100 mg. Only  $29 \pm 3\%$  ( $n = 7$ ) of the remaining load was secreted over the next 80 min. Another group of ticks was injected with 25 µl 1.2% NaCl/100 mg 40–72 h postremoval, and 0% ( $n = 11$ ) of the load was secreted in 3 h. After administration of 53 nM DA/100 mg body wt to these ticks (vehicle being 1 µl 1.2% NaCl/100 mg),  $59 \pm 3\%$  ( $n = 11$ ) of the total load was secreted (double the amount observed for sucrose-injected ticks), the hemolymph volume being reduced to  $29 \pm 2\%$  initial body wt as a result.

The question now arises as to the mechanism(s) employed by the sensory system to monitor hemolymph volume. There are a number of possibilities: 1) an osmotic receptor (when feeding begins, the gut absorbate is hypotonic to the hemolymph; Ref. 8); 2) a receptor that measures internal hydrostatic pressure directly; or 3) stretch receptors in strategically located muscles (as described for the blood-sucking insect *Rhodnius*; Refs. 13, 16). We feel that reduction in osmotic pressure does not play a direct role in triggering salivation. Figure 1 and Tables 1 and 3 show that DW elicited a weak and delayed salivary response, a response that diminished as the osmotic challenge increased. The following two experiments do not support the hypothesis that the tick pos-

sesses a sensu stricto hydrostatic pressure sensor.

1) In the prefed state, the integument of ixodid ticks is quite inflexible (the common name for the family Ixodidae is “hard” ticks). As the tick feeds, however, its integument becomes increasingly more pliable, reminiscent of the plasticization phenomenon, which has been studied in certain insects (15, 23). In the present study, when one administered large volumes of saline (25% body wt) this imposed considerable tautness in the body wall and hence probably an increase in internal hydrostatic pressure. Because the body wall of ticks becomes more compliant as feeding progresses, one might predict that a saline load would cause a smaller increase in internal hydrostatic pressure in larger ticks, and hence a relatively smaller salivary response, were hydrostatic pressure monitored directly by the sensory apparatus. Figure 10 shows precisely the opposite, namely a positive correlation between tick weight and salivary response. The anomalous behavior by replete ticks in Fig. 10 is explicable in terms of degenerative processes known to occur in the salivary glands at this stage of the feeding cycle (5).

2) Ticks, 48 h postremoval, were injected with 25 µl saline/100 mg, a treatment known to elicit no response (Fig. 6). After the ticks were transferred to the observation table for saliva collection, a narrow strip of wood was placed along the upturned ventral surfaces of the ticks. Considerable pressure was applied to, and maintained on, the ticks by encircling stiff elastic bands around the wood strip and observation table. Seven of the eight ticks secreted no saliva within 2.5 h. The eighth tick secreted only 4.3% of the injected load.

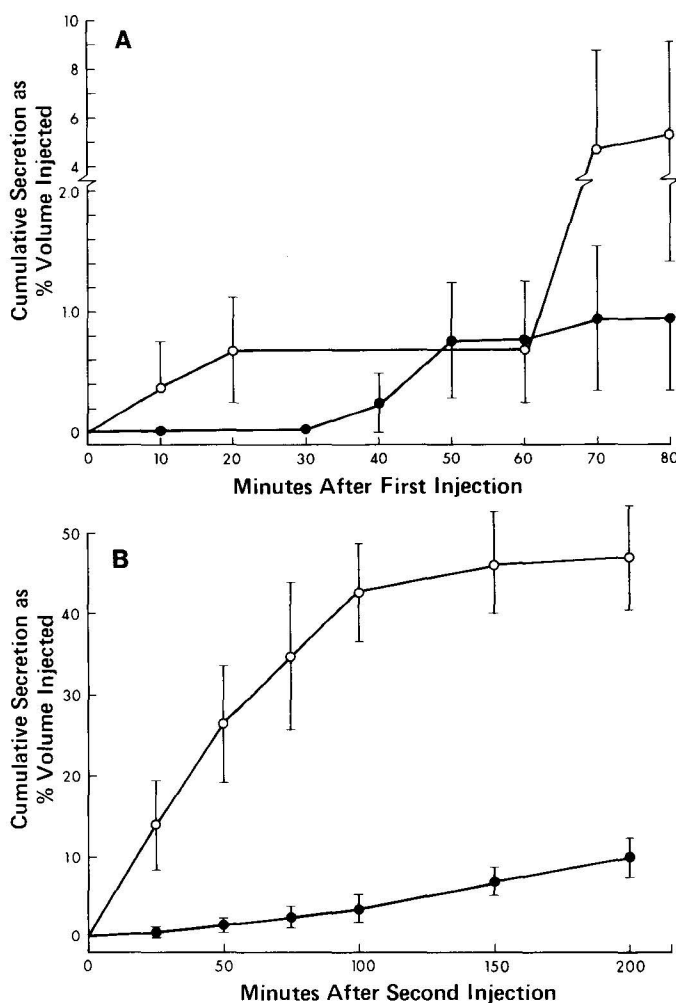


FIG. 5. Effect of reserpine on the salivary secretion induced by 1.2% NaCl (25  $\mu$ l/100 mg body wt). A: reserpine was injected 23 h before injecting the saline. ○, Control ( $n = 6$ ); ●, reserpine, 140  $\mu$ mol/kg body wt, ( $n = 6$ ). There was no significant difference between control and experimental groups. Note that in this experiment even controls hardly responded to this normally effective dose of 1.2% saline (cf. Fig. 1). See text for further explanation. B: ticks from A were injected with a further 25  $\mu$ l 1.2% NaCl/100 mg body wt. ○, Control ( $n = 5$ ); ●, reserpine ( $n = 6$ ). Standard errors are indicated. Differences between control and experimental at each time are highly significant ( $P < 0.01$ ).

## DISCUSSION

Salivary fluid secretion is regulated by a neural control mechanism composed of at least two elements: a cholinergic sensory nerve (or interneuron) impinging on a catecholaminergic secretory nerve. The evidence for this contention can be summarized briefly as follows: whereas catecholamines are potent agonists on isolated glands, the latter are insensitive to cholinomimetic agents (5, 6, 9, 22). Cholinomimetics are, on the other hand, effective agonists in vivo (7, 17), and their action can be markedly attenuated by atropine, reserpine, and guanethidine; the latter drugs do not reduce DA-induced salivation (7). The salivary glands are richly innervated by axons containing dense-core granules (4, 19), terminals of such axons being intimately associated with the cell-type believed to be responsible for the bulk of fluid secretion. Norepinephrine (NE) and DA have been detected in

nervous and salivary tissue of ticks (2, 19, 21). The adult nervous system is a rich source of acetylcholine (25) and finally, choline acetyltransferase, one of the enzymes required for acetylcholine synthesis is present in the nervous system but not in the salivary glands (18).

Until now these pharmacological and morphological data have not been positively linked to some physiological parameter. We show here that injection of fluids into the extracellular space can trigger salivation, even though surprisingly large doses are needed (Fig. 1). The fact that the extracellular space occupies a fairly constant proportion of body weight throughout the feeding period (8) implies that a mechanism exists to enable continual expansion of this space as the tick gorges. In this study we noted definite manifestations of this mechanism, in particular, the reduction in salivary response to saline as a function of the postremoval duration (Fig. 6). It seems reasonable to suppose that the sensory receptor, which somehow monitors hemolymph volume, continually adapts to increasing tick size, and that this adaptation continues even following removal from the host.

The degree to which volume regulation can be correlated to salivation depends on the experimental conditions. DW is probably redistributed fairly rapidly throughout the cellular and extracellular spaces, so that increase in hemolymph volume is buffered to a considerable extent. With regard to saline and sucrose injections, the correlation is clearer. Salivary response to saline was vigorous, but it terminated, on average, before the complete load had been excreted (Fig. 1B). Nevertheless, these ticks did not suffer a corresponding expansion of the hemolymph space (Table 4, *treatment D*). A fraction of the load must have been shunted from the hemolymph to some other compartment, possibly to the cells or possibly to the Malpighian tubules where it was excreted in the urine. It was of considerable interest that a saline challenge offered 2 days after removal from the host was ineffectual in eliciting salivation. The reason was not failure to expand the extracellular space (Table 4, *treatment E*); nor was it due to malfunction of the normal secretory mechanism, since partially fed ticks show no significant variation in their response to DA even up to 5 days postremoval (7). In view of the rapidity with which salivation is attenuated (Fig. 6) we suggest that accommodation of the sensory receptor is responsible for this phenomenon.

Information from different sources also points to the importance of the salivary glands to hemolymph volume regulation. Ticks bearing a heavy infestation of viruslike particles in the salivary gland (so that many of the secretory cells appear damaged) are reported to have much greater hemolymph volumes than do noninfected or lightly infected ticks (20). Teel et al. (27) fed ticks on sheep that were systemically administered the organophosphate insecticide fenthion, and about 30% of these ticks also appeared to suffer an abnormal increase in the extracellular fluid compartment, presumably due to adverse actions of the insecticide on the salivary system.

The dose of saline that elicited the best secretory response (25  $\mu$ l/100 mg body wt) also caused considerable tautness of the abdomen, and we presented some evidence (see above) suggesting that it was not a general

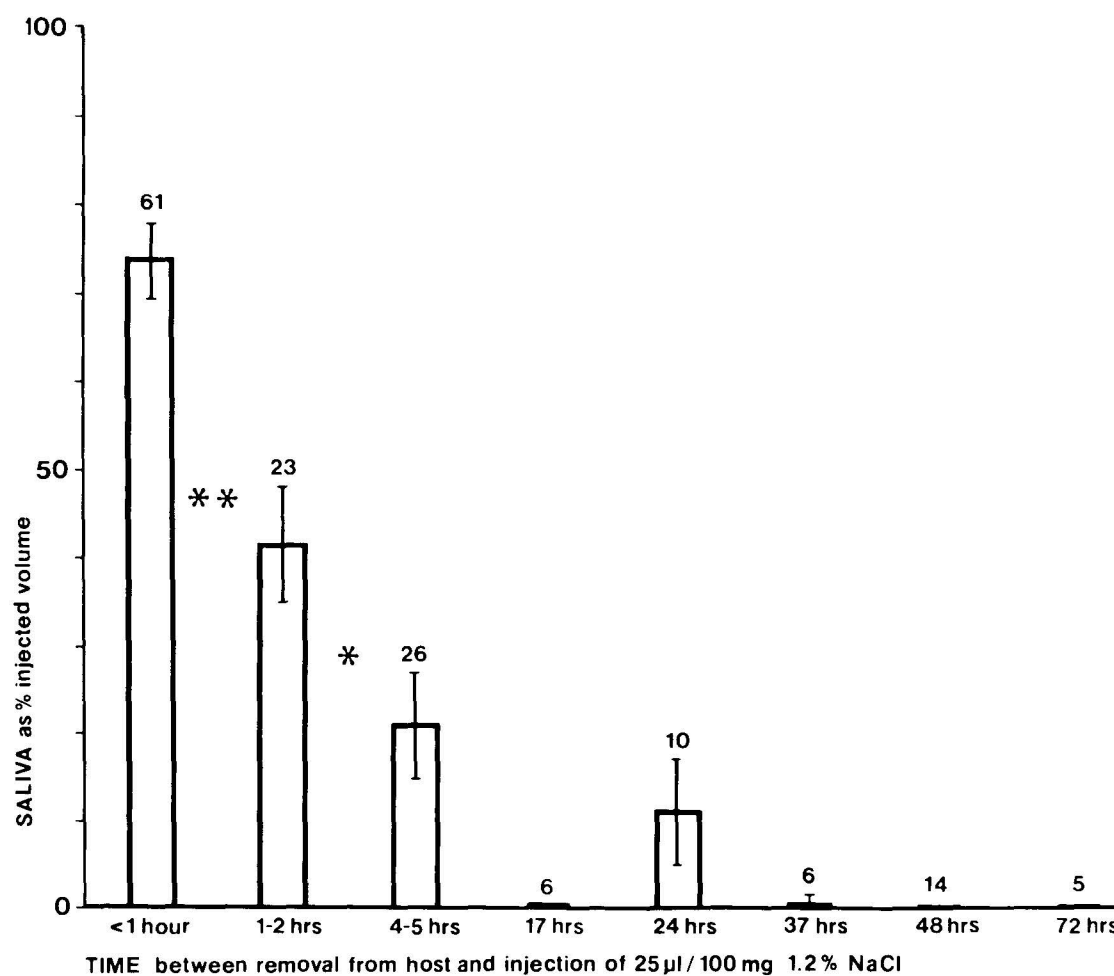


FIG. 6. Secretory response of partially fed ticks injected with 25 µl 1.2% NaCl/100 mg body wt as a function of time between removal of tick from host and injection of saline. Standard errors, *n*, and statistical significance between each pair of bars as for previous figures. Progres-

sive decline in response with time is accompanied by an increase in the latent period between injection of fluid and first appearance of saliva. Latencies (in min) for first 3 groups of ticks are, respectively,  $4.4 \pm 0.8$  ( $n = 44$ ),  $19.0 \pm 5.2$  ( $n = 13$ ), and  $27.7 \pm 6.8$  ( $n = 15$ ).

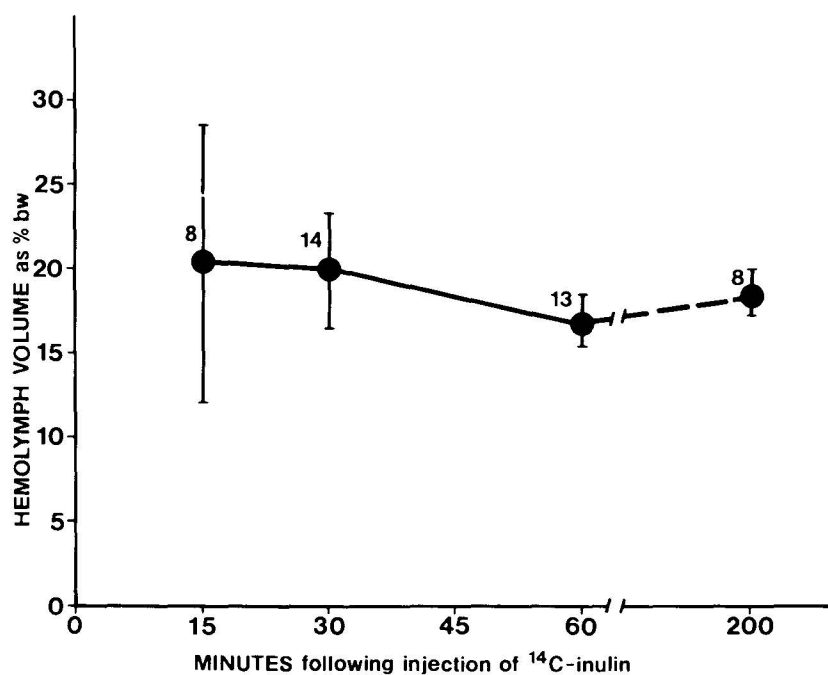


FIG. 7. Equilibration of [<sup>14</sup>C]inulin in hemolymph of partially fed ticks. Hemolymph volume was calculated from dilution of inulin (see METHODS) at various times following injection of tracer. Although there is no significant difference between any of the mean values, variability of data is high prior to 1 h as indicated by standard errors. Thus in subsequent experiments for which hemolymph volume was measured, at least 1 h was allowed for marker to be distributed evenly throughout extracellular space.

increase in internal hydrostatic pressure which constitutes the primary stimulus to secretion. We have no compelling evidence to demonstrate a stretch-receptor mechanism, but the very data that opposed the hydrostatic pressure hypothesis also support the stretch-receptor one; namely, an increase in body wall compliance would permit a given injected volume to stretch certain muscles to a greater extent. In the blood-sucking bug *Rhodnius* stretch of the tergosternal muscles (rather than increased abdominal pressure) appears to initiate diuresis (13, 14).

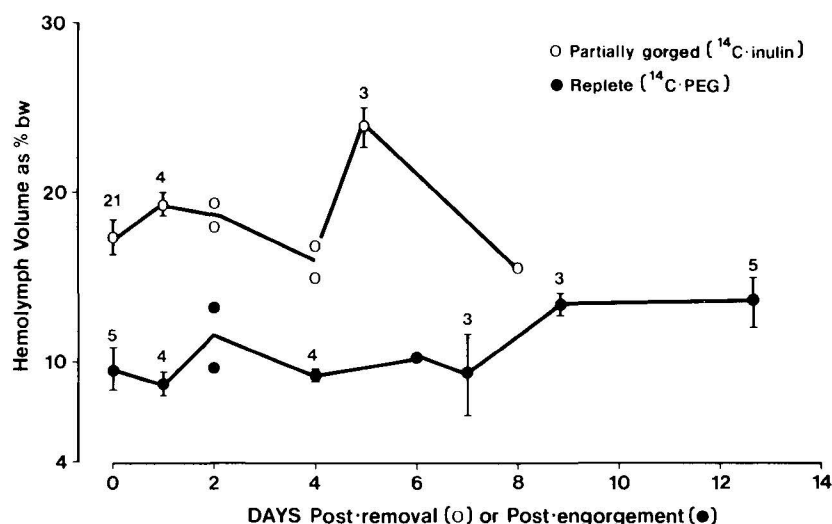
Another potential parameter in the control of salivary fluid secretion is fall in osmotic concentration of the hemolymph. In *D. andersoni*, hemolymph concentration of starved ticks is elevated (approx 525 mosmol/l; Ref. 8). As a result of feeding, the concentration falls and equilibrates to about 375 mosmol/l, a value hyperosmotic to the mammalian blood imbibed (approx 300 mosmol/l). Saliva (hyposmotic) normally appears in feeding ticks about the time that the hemolymph has attained this equilibrated concentration (8). Under normal conditions then, the salivary gland might play a role in osmotic as well as volume regulation. On the other hand, under the present experimental conditions of lowering the hemolymph concentration with DW, the delayed (Table 1) and feeble (Fig. 1) secretory response would have served in osmotic regulation to only a negligible degree.

Atropine only partially inhibited the secretion induced by 1.2% NaCl (Fig. 4). Since atropine at this (145  $\mu$ mol/

TABLE 2. *Inulin and PEG spaces in partially fed and replete ticks*

		Hemolymph, %body wt		
		Mean $\pm$ SE	<i>n</i>	<i>P</i>
A. Partially fed (150-600 mg)	Inulin	17.5 $\pm$ 1.0	21	<0.01
	PEG	23.4 $\pm$ 1.2	11	
B. Replete (< 24 h post-engorgement)	Inulin	7.2 $\pm$ 0.5	4	>0.05
	PEG	9.5 $\pm$ 1.3	5	

For each marker the hemolymph volume of engorged ticks is significantly smaller (as %body wt) than that of partially fed ticks. PEG, polyethylene glycol.



kg) and even lower doses completely abolished the secretory response to injected pilocarpine (7), we propose that the sensory element mediating NaCl-induced salivation is independent of that mediating cholinomimetic-induced salivation. Sucrose-induced and DW-induced salivation were similarly only partially inhibited at best by atropine.

Because the secretory nerve is catecholaminergic, any

TABLE 3. *Effect of injecting DW into hemolymph on salivation and subsequent hemolymph volume using [ $^{14}$ C]inulin as marker*

Treatment	Saliva Secreted, %vol injected	Hemolymph Vol, %body wt
A. Control (no injection)		17.5 $\pm$ 1.0 (21)
B. 5 $\mu$ l DW/100 mg body wt	60 $\pm$ 23 (11)	20.1 $\pm$ 1.3 (11)
C. 5 $\mu$ l DW/100 mg body wt 40-48 h postremoval from host	3.3 $\pm$ 3.3 (11)*	19.2 $\pm$ 2.1 (11)

Values are means  $\pm$  SE, with *n* given in parentheses. DW, distilled water. Although secretion is less in C than in B (0.05 > *P* > 0.01), there is no statistically significant difference between any of the hemolymph volumes (*P* > 0.05). \* Ten of these ticks secreted no saliva; one secreted 36% of the injected load.

TABLE 4. *Effect of injecting isosmotic fluids into hemolymph on salivation and subsequent hemolymph volume using [ $^{14}$ C]PEG as marker*

Treatment	Saliva Secreted, %vol injected	Hemolymph Vol, %body wt
A. Control (no injection)		23.4 $\pm$ 1.2 (11)
B. 10 $\mu$ l 11.2% sucrose/100 mg body wt	22 $\pm$ 12 (6)	31.1* $\pm$ 1.9 (6)
C. 25 $\mu$ l 11.2% sucrose/100 mg body wt	8 $\pm$ 4 (5)	48.6* $\pm$ 4.1 (5)
D. 25 $\mu$ l 1.2% NaCl/100 mg body wt	76 $\pm$ 6 (12)	24.6† $\pm$ 1.2 (12)
E. As D, but 40-48 h postremoval from host	10 $\pm$ 8 (7)	49.3* $\pm$ 2.7 (7)‡

Values are means  $\pm$  SE, with *n* given in parentheses. PEG, polyethylene glycol. By Student's *t* test statistical significance between each treatment and the control volume is as follows: \* *P* < 0.01; † NS, *P* > 0.05. ‡ Ticks in this group received [ $^{14}$ C]inulin as marker; thus hemolymph volume reported here has been increased by 34% (PEG space is 34% larger than inulin space; Table 2) so as to be comparable to other data in this table.

FIG. 8. Hemolymph volume of partially gorged and replete ticks as a function of time postremoval and postengorgement, respectively. Although engorged ticks have significantly smaller hemolymph volumes (expressed as %body wt) than do partially gorged ticks (see also Table 2) there is no significant change in hemolymph volume over time course studied for either group. Where no standard error or *n* is indicated, individual values are shown.

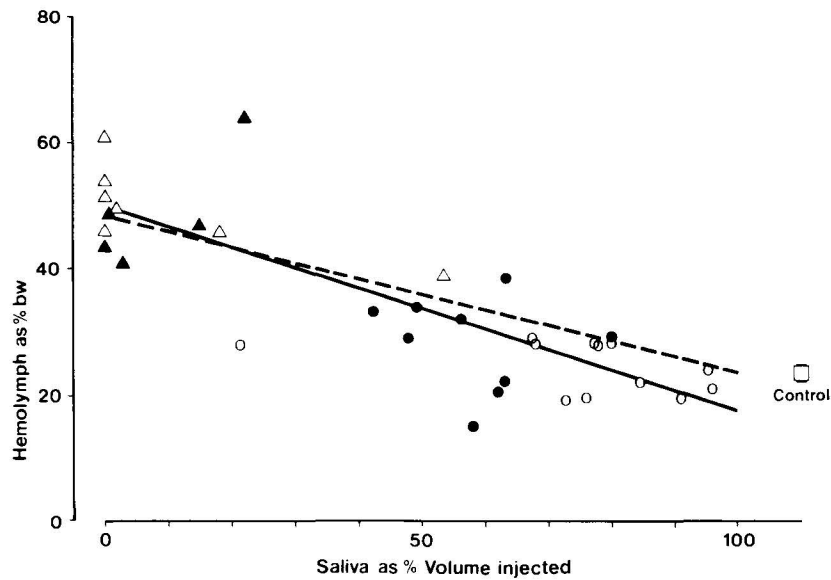


FIG. 9. Hemolymph volume as a function of saliva volume elicited by various challenges. Partially fed ticks were injected with  $25 \mu\text{l}/100 \text{ mg}$  body wt of solutions containing  $6.5 \text{ nCi } [^{14}\text{C}]\text{PEG}/25 \mu\text{l}$ . Treatments were  $\circ$ , 1.2% NaCl injected within 1 h postremoval;  $\triangle$ , 1.2% NaCl injected 40 h postremoval;  $\bullet$ , 1.2% NaCl injected 40-72 h postremoval followed 3 h later with  $53 \text{ nM DA}$  in  $1 \mu\text{l}/100 \text{ mg}$  body wt to induce salivation; and  $\blacktriangle$ , 11.2% sucrose injected within 1 h postremoval. Hemolymph was analyzed for radioactivity after salivary response was recorded. Hemolymph volume for each tick is plotted against volume of saliva secreted. Dashed line ( $Y = -0.25X + 48.4$ ) is the relationship predicted on assumption that salivation is the sole means of removing

injected fluid from hemolymph. Thus, initial hemolymph volume is 23.4% body wt (control ticks ( $\square$ ), data from Table 2, A); after injecting  $25 \mu\text{l}/100 \text{ mg}$  body wt, one can predict from dashed line the subsequent hemolymph volume for salivary responses between 0 and 100% excretion of injected load. Solid regression line for observations is also shown ( $Y = -0.32X + 49.3$ ). Slope is significantly different from zero ( $r = -0.83$  for 33 observations) but is not significantly different from that of dashed line. Nor is the displacement between two lines significant. This was shown by performing a paired  $t$  test between observed values and their corresponding points on dashed line.

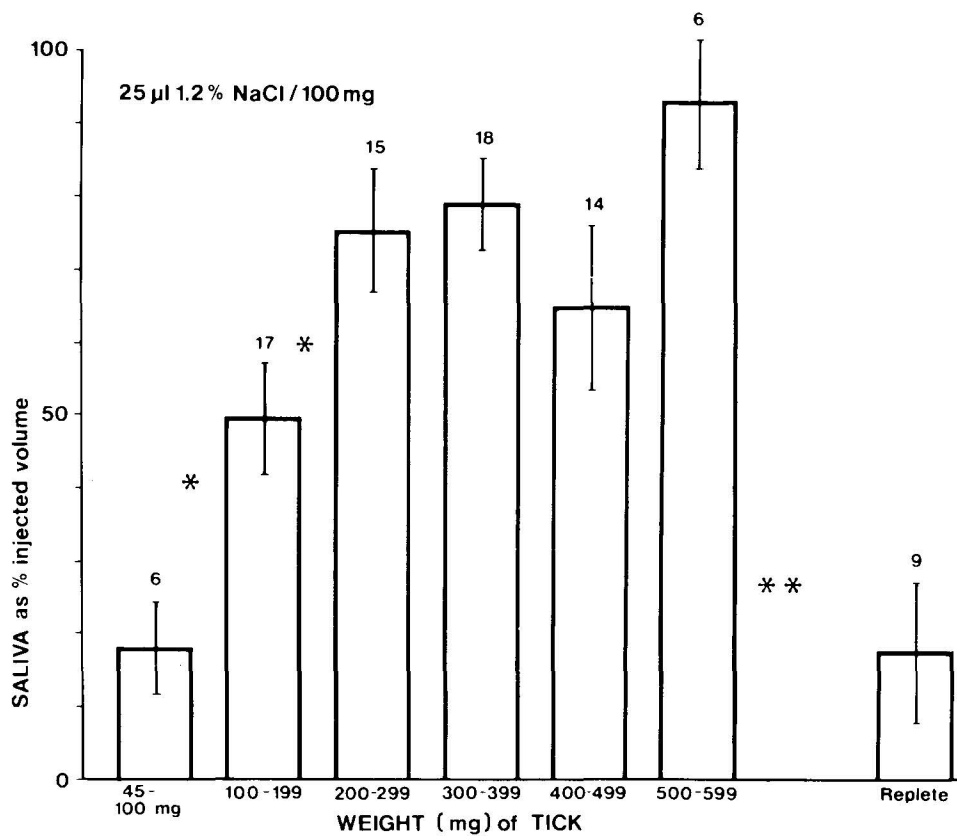


FIG. 10. Relationship between stage of feeding cycle (i.e., weight of tick) and salivary response to an injection of  $25 \mu\text{l}$  1.2% NaCl/100 mg body wt. Asterisks, as in previous figures, denote a statistical difference between bars. There is a clear increased responsiveness to challenge as feeding progresses. Replete ticks show a relatively weak response, presumably because of salivary gland degeneration known to occur at this time (Ref. 5).

stimulus acting via this nerve should be attenuated by reserpine. Consequently, it was not surprising that reserpine inhibited saline-induced salivation (Fig. 5B). The drug does not simply interfere with the secretory mechanism in a nonspecific manner because reserpinized ticks still respond to DA (7).

We thus present in Fig. 11 the simplest neural pathway to account for the present data. The essential feature of the scheme is that two sensory nerves impinge on the secretory nerve. Although we believe that saline-induced salivation is mediated by a noncholinergic (or at least an "atropine-insensitive") pathway, we do not yet have a clue as to the transmitter substance released by this sensory nerve. A catecholamine transmitter would be consistent with the pharmacological data, reserpine acting on both sensory and secretory nerves.

We considered the possibility that the cholinergic pathway might be associated with an osmoreceptor ac-

tivated by reduction in hemolymph osmotic pressure, because in this study atropine significantly inhibited DW-induced secretion (Fig. 4). We then reasoned that if it did not act via a stretch receptor DW-induced (unlike saline-induced) secretion might not be attenuated at 48 h postremoval. Table 3, *treatment C*, shows clearly, however, that this was the case; indeed 10 of the 11 ticks secreted no saliva.

Hyperosmotic loading in the absence of a large fluid volume did not induce salivation (see above). This result was far from surprising because the only appropriate regulatory response by the salivary glands in such a case would be to secrete a hyperosmotic saliva. Isolated glands of *D. andersoni* are unable to secrete a hyperosmotic saliva over a wide range of bathing medium concentrations (10). On the other hand, ticks did drink sufficient DW to dilute the hyperosmotic load to isosmolarity (Fig. 3). Control ticks, however, also drank water; this suggests

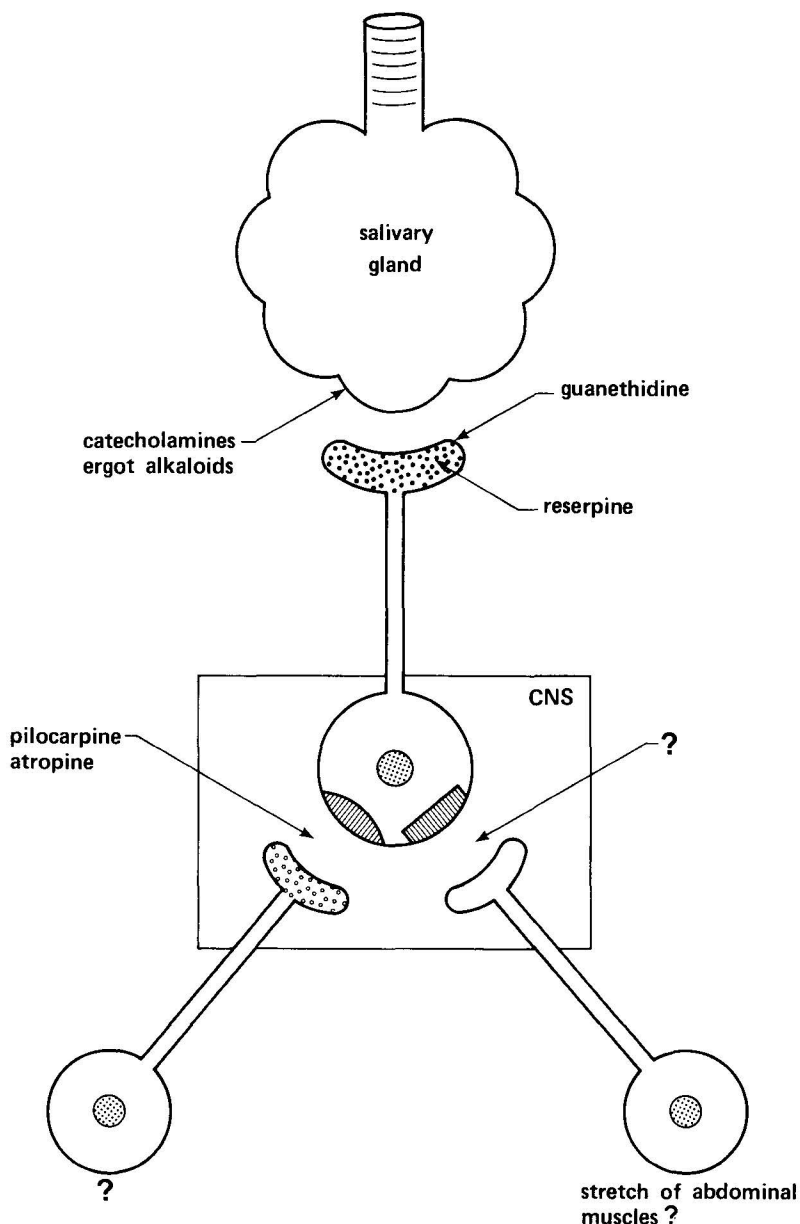


FIG. 11. Simplest schematic diagram to describe control of salivary fluid secretion in ixodid ticks. Salivary gland is innervated directly by a catecholaminergic secretory nerve. This nerve receives input from at least 2 sensory sources. One is a cholinergic nerve, the function of which is unknown; the other is a noncholinergic nerve that relays sensory information deriving from expansion of extracellular space. Transmitter of this nerve is not known. In this diagram we show direct synaptic contact between sensory and secretory elements. In reality there may exist one or more interneurons interposed between sensory and secretory elements, but we have no need to postulate this yet. Probable sites of action of a number of drugs known to affect this system are indicated.

that at least part of the experimentals' drinking behavior was unrelated to the hyperosmotic load. These ticks were all only partially fed, and because ticks feed exclusively on a liquid diet, it is possible that drinking by the controls was related to feeding rather than drinking behavior. In any case, we do not see such a drinking response to be of much potential significance in nature, because the tick once on a host would rarely if ever experience intake of fluid above 300 mosmol/l.

We have already stated our belief that the sensory receptor, in adapting to increasing body size, permits a proportional expansion of the hemolymph compartment. In conclusion, one might ask, What factor determines the limit of body expansion, and thus satiety? We have no evidence relating to this in ticks, but Bennet-Clark (1) has considered this question for *Rhodnius*. He concluded that the relatively inextensible epicuticular layer of the

integument, when fully unpleated, probably produces a backpressure against which the pharyngeal pump can no longer force fluid into the body. This may constitute the signal for cessation of feeding and detachment from the host.

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