Ticks: physiological aspects with implications for pathogen transmission

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Submitted to: *Ticks and Tick-Borne Diseases*, 10 July 2009

Abstract

Ticks have attracted a great deal of scientific attention primarily because of their role as vectors of numerous pathogens. The majority of tick researchers worldwide focus primarily on microbiological and clinical issues relating to these pathogens, and on methods (pesticidal and biological) for controlling tick populations. Unfortunately, it is often forgotten that ticks are also interesting in their own right to the general biologist because of their unusual physiological (and other) adaptations. Here I review some of these adaptations relating primarily to osmoregulation. (1) I outline their ability to take up water vapour directly from the atmosphere, an adaptation that enables them to withstand desiccation for extended periods while unfed and, in the case of larvae and nymphs, following engorgement. (2) I present the remarkable filtration-resorption mechanism of the argasid tick coxal organ, analogous to that of the vertebrate glomerular kidney, that enables them to regulate haemolymph fluid volume and composition following the blood meal. (3) I then turn attention to the salivary glands of female ixodid ticks, which serve the on-host osmoregulatory function in this family of ticks, (4) and I discuss the pharmacological control of salivary fluid secretion. (5) Finally, I link the latter to the mechanism of pathogen transmission by the salivary glands, using the tick-borne Thogoto virus as a specific example.

Introduction

Ticks are interesting largely because of their considerable medical and veterinary importance (Jongejan and Uilenberg, 2004). They are known to transmit numerous arboviruses (eg: tick-borne encephalitis virus and other Flaviviridae, several Reoviridae, Bunyavirida and Iridoviridae), protistans (*Babesia* and *Theileria*), and bacteria (*Rickettsia*, *Ehrlichia*, *Borrelia*) (Sonenshine, 1993). At least one major reason for their efficacy as vectors would be the length of time that they remain attached to their host. Adult female mosquitoes, tsetse flies, the blood-sucking bug *Rhodnius*, the bed bug, *Cimex lectularius* and ticks of the family Argasidae require anywhere from a minute to perhaps an hour to engorge fully to 2 to 15 times their unfed weight. Female ixodid ticks, on the other hand, remain attached to the host for 4 to 14 days, depending on the species and the stage, and feed to about 100X their unfed weight (Kaufman, 2007). But even this impressive figure of 100X underestimates the gluttony of females. The measured engorged weight is only about one-half to one-third the total amount of blood extracted from the host, because a good deal of the water and ions taken in during the meal are excreted by the tick during the feeding period. In brief, such a long period of intimate association with the host is likely to be an important component of their vectorial competence.

Most vectored pathogens are transmitted via the salivary secretions, although in argasid ticks, pathogens may also be transmitted via infected coxal fluid irrigating the feeding lesion made by the mouthparts. Thus, *O. moubata* is more likely to transmit *Borrelia duttonii* via the saliva as nymphs, but more likely to transmit via the coxal fluid when adult (Burgdorfer, 1951). Because *O. hermsi* produces very little coxal fluid, transmission is more likely via the saliva (Herms and Wheeler, 1936).

Because the salivary gland (SG) is the most important route for pathogen transmission by arthropod vectors, it is reasonable to suppose that the volume of saliva secreted into the host would be a major factor determining the efficacy of transmission. For rapid feeders (remaining attached for minutes) the main role of the SGs is to secrete a pharmacological cocktail that facilitates blood flow in the feeding lesion, and so the volume of secreted saliva is probably only in the sub-microlitre to low-microlitre range. For female ixodid ticks, however, the SGs play an additional major role of excreting the excess fluid of the blood meal, and the blood meal is concentrated about two to three-fold (Kaufman and Phillips, 1973a; Koch and

Sauer, 1984). Hence for a large tick like *Amblyomma hebraeum or A. variegatum*, the total volume of secreted saliva during the multi-day sojourn on the host can easily exceed 1 ml.

In this article I shall review some of the physiological processes of ticks that enable them to take in a blood meal and concentrate the nutrient portion by the selective elimination of excess water and ions. The mechanisms are different in the two major families of ticks: salivation in the Ixodidae and coxal fluid excretion in the Argasidae. Because pathogens can be transmitted via both these routes (the saliva being particularly obvious), these physiological systems have relevance for understanding tick-host interaction and pathogen transmission.

Osmoregulation in off-host ticks

When unfed, ticks can be subject to considerable desiccation while questing on vegetation for a suitable host. Whether ticks can drink free-standing water has been a matter for some debate (Knülle and Rudolph, 1982; Needham and Teel, 1986). Kahl and Alidousti (1997) showed, however, that dehydrated *Ixodes ricinus* do not drink free water when presented with it. So how do ixodid ticks survive off host for numerous months? First they share with some insects the property of having an integument that is relatively impermeable to water. But also like some insects, they exhibit the remarkable ability to sorb water vapour directly from the atmosphere when at least two conditions are met: they must be dehydrated to a certain degree, and the relative humidity (RH) of the atmosphere must exceed a certain critical value. This so-called ‘critical equilibrium humidity’ (CEH) is a species-specific value, which typically spans the range of 80-95%, but in some insects (eg the Thysanura), the CEH can be as low as 50% (Edney, 1977). In ticks, the values range from about 75–95%, depending at least on the species and the developmental stage (Needham and Teel, 1986; Gaede and Knülle, 1997; Yoder et al., 2006). Although ticks quest on vegetation during the active periods of their preferred hosts, at other times they descend to a microhabitat at the base of the vegetation where the RH is above the CEH.

Although water vapour uptake in ticks has been best studied in the unfed state, fully engorged larval and nymphal ticks can also actively sorb water vapour from an unsaturated atmosphere. Kahl and Knülle (1988) were the first to show this in *I. ricinus*, *I. scapularis* and *Haemaphysalis punctata*, though not in engorged nymphs of *Hyalomma anatolicaum excavatum*. In the wild, engorged larvae and nymphs require several months to moult to the

subsequent stage, perhaps explaining why the water vapour uptake mechanism has been retained in engorged immature stages. Kahl et al. (1990) demonstrated clearly that the ability to sorb water vapour by engorged nymphs is lost abruptly at the time of apolysis (portion of the moulting cycle at which separation of the cuticle from the underlying hypodermis occurs). Prior to this time, the agranular acini (type I) have a normal appearance under the light microscope, whereas the granular acini (types II and III) degenerate within a few days post-engorgement (Kahl et al., 1990) by a process of programmed cell death (L'Amoreux et al., 2003, Furquim et al., 2008). At the time of apolysis, however, the agranular acini also degenerate, as a prelude to their redevelopment for the next stage. The fact that water vapour uptake can occur even when the granular acini have degenerated, but not once the agranular ones have degenerated, strongly indicates that the agranular acini alone are responsible for the water vapour uptake mechanism (Kahl et al., 1990).

The situation for feeding adult females is less clear. Histological data would suggest that water vapour uptake should occur, because the agranular acini do not degenerate until near the end of the oviposition period (Kahl et al., 1990). However, Lees (1946) found no indication for active vapour uptake in engorged *I. ricinus* females, although he did not use constant recording of tick body weight, a technique that became standard only much later. Several later studies entertain the possibility that engorged or partially fed females of several species may retain the ability to sorb water vapour, at least during the pre-oviposition stage (see Needham and Teel, 1986 for a discussion), though this is not the case in *I. ricinus* (Kahl and Knülle, 1988). However, it is possible that any putative sorption of water is masked by a substantial increase in tracheal water loss, because the spiracles do open and close more frequently following engorgement (discussed by Kahl and Knülle, 1988). Hence, engorged female *I. ricinus*, lose about 1 mg/day during the preoviposition period, whereas the rate of water vapour uptake in an unfed, moderately dehydrated female is no more than 0.15 mg/day (Kahl and Knülle, 1988). After oviposition begins, the active uptake of water vapour by gravimetric methods would be very difficult to detect relative to the much greater loss of weight due to oviposition. The question regarding active water uptake by engorged females will remain open until techniques are developed that can measure water exchange per se against a background of much larger weight losses due to general metabolism and oviposition.

To take up water vapour from the atmosphere at RHs below 100% implies the expenditure of metabolic energy. The rectum is the site of uptake in the Thysanura, and the rectal epithelium shows an incredible density of closely packed mitochondria (Edney, 1977). The cellular mechanisms responsible for the phenomenon are not well known, however. In ticks, water vapour uptake occurs via the oral cavity, and the SGs play the major role (Rudolph and Knülle, 1974). When a dehydrated tick finds itself in an atmosphere above the CEH, it secretes a tiny droplet of saliva onto the mouthparts. This saliva is hygroscopic, so atmospheric water vapour condenses on it. The tick then swallows the now enlarged droplet, secretes a fresh aliquot of saliva and the process repeats until the tick has achieved a normal level of hydration. As already mentioned, this so-called 'rehydration saliva' is assumed to be secreted by the type I acini of the SG. These acini have the ultrastructural properties characteristic of a tissue functioning in active transport processes (Fawcett et al., 1986). It is assumed that some (still unknown) organic solute is responsible for the hygroscopic properties of this saliva (Knülle and Rudolph, 1982; Needham and Teel, 1986).

Osmoregulation in on-host ticks

Because of their large surface-to-volume ratios, ticks would succumb to desiccation fairly rapidly if it were not for the relatively impermeable integument and the water vapour uptake mechanism just described. When feeding on a host's blood, however, water is in super-abundance. Mammalian blood is also about 20% hyposmotic to the tick's (*Dermacentor andersoni*) tissue fluids (Kaufman and Phillips, 1973a). So how does the tick maintain its tissue fluids hyperosmotic to the huge volume of hyposmotic blood passing through the body? Blood-sucking insects use their Malpighian tubules to excrete the excess fluid of the blood meal and maintain osmotic equilibrium (Maddrell, 1981). Although argasid and ixodid ticks use their Malpighian tubules for nitrogen excretion as do insects, the two major tick families have each developed a unique system for dealing with the excess water of the blood meal; argasid ticks use their coxal organs and ixodid ticks use their SGs. Use of the SGs for osmoregulation by ixodid ticks refers only to the female.

Males imbibe a very small meal and so the osmotic stress they experience is not large, and to my knowledge, nobody has yet explored osmoregulation in the feeding male. However, during feeding, the male SG does not develop the ultrastructural characteristics typical of the female. Coons and Kaufman (1988) demonstrated that the feeding female (*A. hebraeum*) releases a factor

into the HL that stimulates SG development. They did this by transplanting the SGs from unfed females into the haemocoel of partially fed females that were subsequently allowed to feed to engorgement. The transplanted glands developed the ultrastructural changes characteristic of feeding females, suggesting that normal development of the SG is due to a specific hormone. SGs from unfed males transplanted to feeding females developed to the same extent as transplanted SGs from unfed females. It is fascinating that, “over evolutionary history, male tissue may have retained sensitivity to a factor [that] it normally would never experience” (Coons and Kaufman, 1988).

Argasid ticks

The coxal organ of argasid ticks (Fig. 1) has a structure somewhat similar to a vertebrate filtration-resorption renal system. Ultrastructure of the filtration membrane of the coxal organ bears a striking resemblance to that of the filtration membrane of a vertebrate glomerular nephron (Fig. 2A; Hecker et al., 1969), undoubtedly due to evolutionary convergence. Ultrastructure of the coxal tubular cells (Fig. 2B) likewise shows many characteristics of other resorptive epithelia, such as the proximal convoluted tubule of the glomerular nephron (S.E. Kaufman, 1971). Hecker et al. (1969) state that there are two distinct populations of tubular cells: ‘short cells’ (with long microvilli) and ‘tall cells’ (with short microvilli). The short cells are stated to occur in the proximal part of the tubule (with respect to the junction with the filtration membrane) and the tall cells occur in the distal region, although the proportional length of the tubule occupied by each cell type was not stated. S.E. Kaufman (1971) did not specify which of the two cell types is depicted in her thesis (the cell type reproduced here in Fig. 2B), although based on the prominent microvilli, it is likely to be a short, proximal cell.

There is also direct physiological evidence for filtration/resorption.

1) One of the general characteristics of filtration systems is that the rate of fluid production is much more rapid compared to that of a secretory epithelium. A coxal organ of *O. moubata*, weighing only ~0.3 mg, produces coxal fluid at a rate of ~1.5 µl/min (S.E. Kaufman, 1971). A SG of an *A. hebraeum* female, however, weighing about 5 mg (though a significant proportion of that 5 mg would not be involved in fluid secretion), secretes saliva at a maximum rate of only ~0.5 µl/min.

So on a tissue weight basis, the argasid coxal organ produces fluid at ~25-50 times the rate of an ixodid female SG.

2) In the mammalian glomerular afferent capillary, the hydrostatic pressure is estimated to be ~60 mm Hg (Davson and Segal, 1975). In *O. moubata*, the hydrostatic pressure in the HL, during the time that coxal fluid excretion occurs, is ~30-50 mm Hg, with spikes occurring up to 100 mm Hg (Kaufman, Kaufman and Phillips, 1982). This is well within the range of systolic pressures reported for fishes that have glomerular kidneys (Jones and Farrell, 1992).

3) Filtration membranes are generally permeable to most molecules smaller than plasma proteins. Thus the filtration membrane of the coxal organ of *O. moubata*, like that of the vertebrate glomerulus, is freely permeable to inulin (MW ~5000) (Kaufman, Kaufman and Phillips, 1982), whereas the SG of *A. hebraeum* is essentially impermeable to this polysaccharide (Kaufman Aeschlimann and Diehl, 1980).

4) Metabolites, such as glucose, are normally almost completely reabsorbed from the glomerular filtrate of mammals by the proximal convoluted tubule. The glucose transport mechanism of this epithelium can be blocked by phlorhizin (Pitts, 1963). Likewise, the coxal fluid of *O. moubata* normally contains very little, if any, glucose, but when ticks were pre-injected with phlorhizin, the glucose concentration of the coxal fluid rose to a level similar to that of the HL (Kaufman, Kaufman and Phillips, 1982).

All this indicates that coxal fluid production in *O. moubata* occurs by a filtration-resorption mechanism.

Ixodid ticks

Blood sucking insects and argasid ticks begin excreting the excess fluid of the blood meal shortly before or soon after detachment. But in most ixodid ticks, there is little if any elimination of fluid to the exterior following detachment. Lees (1946) proposed that elimination of fluid in *I. ricinus* might be by transpiration from the integument, because he demonstrated that the permeability of the integument to water increases substantially at the surface temperature of the host sheep. However, because *I. ricinus* ticks were able to regulate their body water content even when feeding

in a microenvironment at near 100% RH, Lees himself was aware of a critical weakness of this hypothesis.

Gregson (1967) was the first to hypothesize that salivation might serve an osmoregulatory function during the blood meal. His hypothesis was based on direct observations of the mouthparts of ticks (*D. andersoni*) while they were feeding on a host. Direct experimental support for this hypothesis was provided by Tatchell (1967a), working with the Australian cattle tick, *Rhipicephalus* (=*Boophilus*) *microplus*. Tatchell injected tritiated water into the haemocoel of eight ticks still attached to a host on a given afternoon, but expected to engorge the following morning. He recovered almost 75% of the total radioactivity injected into the ticks. Most of it was recovered from five ticks that had not detached spontaneously overnight, and from host body fluid and urine. Very little was found in the three ticks that had engorged. The easiest way to explain very little radioactivity in the engorged ticks, most of it in the unengorged ticks, and a significant amount of it in host body fluid and urine, is by the direct passage of water from tick to host via the saliva.

Tick SG physiology relating to osmoregulation and HL volume regulation

The SGs are prominent organs situated bilaterally, and extending from the capitulum to the spiracles in the opisthosoma. The SGs comprise three distinct types of acini in females (types I, II and III), and a fourth in males (Fawcett et al., 1981a, 1981b). The relationship of the SGs to the transmission of *Theileria parva* (etiologic agent for African East Coast fever) is presented by Fawcett et al. (1982). An excellent review of all this can be found in Fawcett et al. (1986) and Coons and Alberti (1999).

One can stimulate salivation in vivo in argasid ticks (Howell, 1966) and in ixodid ticks (Tatchell, 1967b, Purnell et al., 1969) by topical application of the cholinomimetic drug, pilocarpine (PC). The catecholamines, adrenaline, noradrenaline and dopamine (DA), also stimulate salivary fluid secretion in vitro (Kaufman and Phillips, 1973b, Kaufman, 1976), with DA being about 20 times more potent than adrenaline or noradrenaline (Kaufman, 1976, 1977). However, PC does not stimulate salivary fluid secretion in vitro (Kaufman and Phillips, 1973b; Kaufman, 1978). Moreover, If the major nerves to the SG are cut, the in vivo response to PC is virtually abolished, whereas that to DA is not significantly inhibited (Kaufman and Harris, 1983). All this indicates that DA, and presumably the other catecholamines, act via a

receptor on the fluid secretory cell, whereas PC acts indirectly by stimulating a secretomotor nerve to the SG.

The earliest investigations about the physiological role of the SGs during feeding relate to the role of ions in SG function (Tatchell, 1969). In *D. andersoni*, the concentrations of Na^+ and K^+ in the saliva are similar to those in the HL, whereas the Cl^- concentration of saliva is significantly higher than that of the HL. Saliva is also about 5% hyposmotic to the HL (Kaufman and Phillips, 1973a). The secretion of a large volume of slightly hyposmotic saliva would account for how the tick maintains a HL osmotic pressure higher than that of the blood meal imbibed. Kaufman and Phillips (1973c) reported that the transacinar electro-potential difference is ~35 mV, lumen negative to bathing medium. Moreover, replacing Cl^- in the bathing medium with a non-halide anion markedly, and reversibly, inhibits fluid secretion. All this demonstrates that fluid secretion across the acinus is probably generated by the active transport of Cl^-. However, the Na^+ and K^+ requirements for fluid secretion are also highly specific. With Na^+ as the major cation in the medium bathing isolated SGs, salivary fluid secretion in vitro was only about 20% maximal. But adding 10 m.equiv. of K^+ resulted in a maximal fluid secretory rate; Raising the K^+ concentration beyond 10 m.equiv/l led to a progressive inhibition of fluid secretion (Kaufman and Phillips, 1973c). Such a profile suggested that Na/K-ATPase is an important component of the salivary fluid secretory mechanism. This is supported by the fact that salivary fluid secretion in vitro is completely blocked by micromolar concentrations of the Na/K-ATPase inhibitor, ouabain (Kaufman and Phillips, 1973c). The ontogeny and basic properties of this tick SG Na/K-ATPase have been described (Kaufman, Diehl and Aeschlimann, 1976; Rutti et al., 1980).

Kaufman, Aeschlimann and Diehl (1980) demonstrated that the SGs play a major role in HL volume regulation. They injected large volumes (up to 50% body weight) of various isosmotic solutions (NaCl, glucose, sucrose, urea) or distilled water into the haemocoel of partially fed *A. hebraeum*, and monitored subsequent saliva production. NaCl was the most effective salivary fluid stimulant, with 75% of the injected load being secreted by the SGs within an hour post injection of a volume equivalent to 25% bw. Moreover, there was a quantitative correlation between the volume of saliva secreted and the ultimate HL volume achieved. Using ^{14}C-labelled inulin as a HL space marker, Kaufman and Phillips (1973a) demonstrated in *D. andersoni* that HL volume is maintained at a constant proportion of body weight (about 23%) throughout a normal feeding cycle. Taking the data on injected fluids, and

using ^{14}C-polyethylene glycol as a space marker, Kaufman, Aeschlimann and Diehl (1980) plotted the resulting HL volume (as % bw) as a function of saliva secreted (as % volume injected into the HL). Figure 3 shows a very close correspondence between the linear regression curve through the data points and the theoretical line predicted on the assumption that salivation is the *only* means for removing injected fluid from the HL.

What is the nature of the sensory system that stimulates salivation as feeding progresses? Three possibilities are: (A) an osmoreceptor that detects the continual dilution of the tick's body fluids as more hyposmotic blood is imbibed, (B) a hydrostatic pressure sensor (internal hydrostatic pressure should increase, at least transiently, as feeding progresses) and (C) stretch receptors in strategically placed muscles — perhaps the dorso-ventral muscles in the opisthosoma. Because injecting distilled water into the HL (mentioned above) resulted only in a weak and delayed salivary secretory response, Kaufman, Aeschlimann and Diehl (1980) suggested that if the tick does possess osmoreceptors, they probably play only a minor role in stimulating salivation. To test hypothesis B, the latter authors increased the internal hydrostatic pressure in some experimental ticks by mechanical pressure on the opisthosoma. Most of these ticks secreted no saliva and one secreted only a very small volume within 2.5 hours. The third hypothesis is the normal sensory mechanism used to trigger Malpighian tubule secretion in the blood-sucking insect, *Rhodnius* (Maddrell, 1964). However, attempts in my laboratory to test this directly in *A. hebraeum* gave ambiguous results (Patriquin, 1991).

Pharmacology of the sensory system controlling salivary fluid secretion

The innervation to the SG has been well documented (Saito, 1960; Binnington, 1981; Shoukrey and Sweatman, 1984). Dopamine stimulates salivary fluid secretion in vitro, suggesting that it acts directly via receptors on the SGs, and that it is the neurotransmitter. The DA receptor of the SG is of the D_1 subtype (Schmidt et al, 1981) and the SG also has a DA-sensitive adenylate cyclase (Schmidt et al., 1982). Moreover, there is a high concentration of DA in the synganglion and in the SGs of ticks, although a substantial proportion of DA in the SG is probably contained in the granule cells rather than in dopaminergic nerve terminals (Kaufman, Sloley et al., 1999). DA associated with the granule cells is assumed to be secreted into the saliva, but if so, the function of this DA is not known.

Because cholinomimetic drugs, such as PC, have no agonist activity when applied directly to isolated SGs, the implication is that cholinomimetics act somewhere in the sensory pathway leading to the secreto-motor nerve. The cholinergic receptor via which PC acts is probably of the muscarinic type, because PC-induced salivation can be blocked by atropine (Kaufman, 1978). If the action of PC is mediated via the dopaminergic secreto-motor nerve, one would expect reserpine and guanethidine to inhibit PC-induced secretion without inhibiting the action of DA. This is because a major effect of guanethidine (in mammals) is to inhibit the release of the catecholamine neurotransmitter, and a major effect of reserpine is to deplete catecholaminergic nerves of the neurotransmitter (Nickerson and Collier, 1975). Pretreatment with guanethidine and reserpine had the expected effect in ticks subsequently injected with PC (Kaufman, 1978). Along with the demonstration by Kaufman and Harris (1983) mentioned earlier, that cutting the major SG nerves inhibits the action of PC but not DA, these pharmacological data support the hypothesis that PC stimulates salivation via the secreto-motor nerve to the SG.

When Kaufman, Aeschlimann and Diehl (1980) discovered that increasing HL volume with large volumes of isosmotic saline stimulates salivation, it was obvious to test whether atropine (a potent inhibitor of PC) would block saline-induced secretion. However, the inhibitory effect was minimal when they used a dose of atropine that completely blocked the action of PC. Thus there appears to be at least two sensory pathways impinging on the SG: one of them is a muscarinic (i.e., atropine-sensitive) cholinergic nerve, the physiological function of which is unknown. The other is part of a sensory pathway that monitors HL volume, the neurotransmitter of which is unknown.

Pharmacological control of fluid secretion: Receptors associated with the fluid secretory tissue

Catecholamines

The fact that DA was about 20X more potent than noradrenaline or adrenaline in stimulating salivary fluid secretion in vitro (Kaufman, 1976) suggested that the catecholamine receptor on the SG might be a DA-receptor. A more extensive series of dose-response experiments conducted in vitro supported this hypothesis. In what follows, the drug concentration resulting in 50% maximum response is shown in parentheses. Among numerous catecholamines and catecholamine-like drugs, DA was the most potent (~30 nM),

followed by noradrenaline and adrenaline (~500 nM). The remaining agonists were all far less potent still: isoproterenol (~10 µM), norphenylephrine and beta-phenylethylamine (~100 µM), phenylephrine (> 100 µM), tyramine and DOPA (~300 µM) and octopamine (~1000 µM) (Kaufman, 1977). Several drugs showing reasonable specificity for mammalian DA-receptors were also reasonably potent agonists of salivary fluid secretion: epinine (~100 nM), 6-hydroxydopamine (~300 nM) and apomorphine (~30 µM). Ergot alkaloid drugs have a very wide spectrum of activity (agonism and antagonism) on numerous catecholamine and tryptamine receptors (Berde and Schild, 1978). Ergonovine and ergotamine were agonists of tick salivary fluid secretion at least as potent as DA (Kaufman, 1977), again lending support to the DA-receptor hypothesis. But see more on ergot alkaloids in the next section for a different interpretation.

Results from testing a number of catecholaminergic antagonists also supported the hypothesis of a DA-receptor. In brief, the α-adrenergic antagonists (phenoxybenzamine, phentolamine), and β-adrenergic antagonists (propranalol, dichloroisoprenaline) were ineffective at concentrations below 100X - 1000X the concentration of DA, and thus Kaufman (1977) considered their antagonism to be non-specific. A number of recognized DA-antagonists were also ineffective at blocking the action of DA below very high concentrations (chlorpromazine, α-flupenthixol, pimozide, spiperone; Kaufman, 1977) and sulpiride (Kaufman and Wong, 1983). However, (+)-butaclamol, the well known DA-anatagonist, was an effective and potent antagonist (K_i = 60 nM) of DA-induced salivary fluid secretion, and its antagonism was surmountable on raising the DA-concentration; (-)-butaclamol was ineffective on the tick SG, as it is at mammalian DA-receptors (Kaufman and Wong, 1983).

Ergot alkaloids

If the ergot alkaloids stimulate fluid secretion via a DA-receptor, one would expect (+)-butaclamol to inhibit their action on the SG, but it did so only weakly (Kaufman and Wong, 1983). On the other hand, sulpiride (a DA-receptor antagonist in mammals) was an effective antagonist of ergot alkaloid-induced salivary secretion, but not of DA-induced secretion (Kaufman and Wong, 1983). These surprising results demonstrated that ergot alkaloids and DA do not stimulate salivary fluid secretion via a common receptor. Because ergot alkaloids are not endogenous to any animal, the question arises as to why there is a receptor for this family of drugs in the tick SG. One assumes it is acting at a (non-DA) receptor for a hormone or neurotransmitter endogenous to the

tick. Ergot alkaloids also bind to 5-hydroxytryptamine (5-HT) receptors in numerous organisms (Berde and Schild, 1978), but 5-HT only occasionally stimulates salivary fluid secretion in ticks, and then at concentrations only in the millimolar range (Kaufman and Phillips, 1973b; Kaufman and Minion, 2006). So ergot alkaloids are unlikely to stimulate salivary fluid secretion via a 5-HT receptor. However, at least we now have a better understanding of the pharmacological actions of 11 ergot alkaloids on isolated tick SGs (Kaufman and Minion, 2006). Four of them (dihydroergotamine, ergonovine, methylergonovine and α–ergocriptine) are full agonists, resulting in a maximal response similar to that of DA. Three of them (ergocornine, methysergide, bromocriptine) are partial agonists, producing a maximal response only 22-50% that of DA. Three of them (ergocorninine, ergocristinine and ergocristine) were denoted as “incomplete agonists”, showing no plateau response at the highest concentration tested, and one of them (ergothioneine) had no agonist activity up to a concentration of 1 mM. The partial agonists, methysergide and bromocriptine, were also competitive antagonists at the ergot alkaloid receptor, but not at the DA-receptor (Kaufman and Minion, 2006). So the latter study has offered us some additional pharmacological tools with which to explore the DA- and ergot alkaloid-pathways independently from each other.

A great deal of work has been done on the intracellular signaling pathways mediating the action of DA-receptor stimulation (though not, unfortunately, of ergot alkaloid-receptor stimulation), beginning with the demonstration by Needham and Sauer (1975, 1979) of the importance of calcium and cyclic AMP, and by Schmidt et al. (1981, 1982) that DA-induced fluid secretion is mediated by a DA-linked adenylate cyclase. Since then, much more has been learned about the roles of other signaling pathways. This fascinating body of literature is beyond the scope of this review, but a diagrammatic summary is shown in Fig. 4, and further details are reviewed by Sauer et al. (1995; 2000) and Bowman et al. (2008). Recently, it has been demonstrated that the cAMP and cGMP pathways in the SG are more intimately intertwined than hitherto suspected (Bladow et al., 2009).

Butyrophenone drugs

Butyrophenones are specific and potent inhibitors of mammalian CNS DA-receptors (Seeman, 1977). But spiperone and pimozide were devoid of an inhibitory effect on tick SGs. In fact, they actually potentiated the action of DA, even though they did not stimulate fluid secretion on their own (Kaufman, 1977). This potentiation was not merely a conventional

leftward shift of the DA dose-response curve, but a more unusual type involving an increase in the maximum response to agonist (see Barnett et al., 1968; Reiffenstein, 1968) by up to 100%.

The potency of spiperone's potentiation of DA on the tick SG is extraordinary. With 0.23 µM DA stimulating a near-maximal fluid secretory response (~80%), adding just 10^{-14} M spiperone resulted in a secretory rate of ~120% maximum (Wong and Kaufman, 1981)! The total dose-response curve for spiperone was complex, however. There was a plateau effect at ~120% maximum between 10^{-14} M and 10^{-11} M. Then the potentiation was further augmented to ~150% between 10^{-10} M and 10^{-9} M. The degree of potentiation increased yet again to ~180% at 10 µM (Wong and Kaufman, 1981). Such an extensive and complex dose-response profile suggests that the specific mechanism of potentiation may change according to the specific concentration range, though this has not been explored further in tick SGs. Nine other butyrophenone drugs, assayed at 10^{-9} M and 10^{-6} M, likewise potentiated the effect of DA to a greater or lesser extent (Wong and Kaufman, 1981).

The potentiation of DA by spiperone can be blocked by sulpiride, the same antagonist of ergot alkaloids (but not DA) on the tick SG (Kaufman and Wong, 1983). Thus, notwithstanding that the butyrophenone drugs are antagonists of mammalian CNS DA-receptors, they do not act directly at the tick SG DA-receptor, or at least not at the DA-binding site.

Gamma-aminobutyric acid (GABA)

Because the butyrophenones are synthetic drugs, their pharmacological action probably reflects the existence of some endogenous neural or hormonal system that modulates DA effects in the tick. Moreover, because butyrophenones are known only as antagonists, how might one explain a potentiating effect from an antagonist drug on this endogenous system? Lindsay and Kaufman (1986) tested the following model: Suppose that the tick SG normally receives inhibitory innervation. When the gland is dissected out for in vitro experiments, perhaps there is a tonic release of an inhibitory neurotransmitter from the nerve terminals. So when the glands are bathed in a supra-maximal concentration of DA, the secretory response recorded as 'maximal' is, in reality, sub-maximal because of this braking action of the inhibitor. Spiperone could conceivably block the release of this inhibitory transmitter or block its receptor, thus causing an apparent potentiation.

GABA is a widespread inhibitory neurotransmitter. Lindsay and Kaufman (1986) designed an experiment to test whether high concentrations of GABA would inhibit the effect of DA on the tick SG and whether spiperone would inhibit the inhibitory effect of GABA. However, GABA itself potentiated the action of DA as spiperone does. So although the hypothesis was not supported, the data revealed a novel, non-inhibitory, effect for a classical inhibitory neurotransmitter. A dose-response trial for GABA demonstrated a threshold potentiating effect at <1 µM, and a maximum response at 100 µM (Lindsay and Kaufman, 1986). The potentiation by GABA was inhibited by the well known GABA antagonists, picrotoxin and (-)-bicuculline, as well as by sulpiride. The effect of spiperone could also be inhibited by picrotoxin and bicuculline, confirming that GABA and spiperone do indeed potentiate DA via a common receptor. Because the GABA-mimic, muscimol, but not baclofen, potentiated DA-induced secretion, this indicates that the tick GABA receptor is of the $GABA_A$ type (Lindsay and Kaufman, 1986).

Lucien et al. (1995) assayed a variety of tick tissues throughout the feeding cycle and post-engorgement period for endogenous GABA (and some other amino acids) using HPLC. GABA was detected in all tissues sampled, and the titres fluctuated significantly throughout the feeding/post engorgement periods. Particularly noteworthy was that the SG titres of GABA were relatively low (15–110 nmol/g) during most of the feeding period, but rose substantially (to 685 nmol/g) on the day of engorgement. Does this high GABA titre in the tissue correlate with a noticeably higher rate of salivary fluid secretion? Apparently so: isolated SGs from day 0 engorged ticks exposed to 10 µM DA secreted 8.4 mg fluid/gland/10 min, whereas glands taken from ticks at the end of the slow phase of engorgement and exposed to 10 µM DA secreted only 6.3 mg fluid/gland/10 min. Moreover, when SGs taken from engorged ticks were exposed to 10 µM DA plus 100 µM picrotoxin, the secretory response fell to 5.9 mg/gland/10 min. Picrotoxin did not, however, reduce the effect of DA on SGs taken from partially fed ticks (6.7 mg/gland/10 min in DA plus picrotoxin vs 6.3 mg/gland/10 min in DA alone; Lucien et al; 1995).

Figure 5 summarizes what we currently know about the sensory pathways impinging on salivary fluid secretion, as well as the receptors on the SG mediating fluid secretion. As reviewed here, some of the pharmacological properties of the tick SG receptors are significantly different from what one would predict about catecholamine receptors in mammalian models and even in some insect models. Be aware, however, that our studies on

the DA responses were conducted without blocking the ergot alkaloid and GABA receptors with sulpiride, and our studies on the ergot-alkaloid and GABA responses were conducted without blocking the DA receptor with butaclamol. Now that we are aware of a few selective antagonists for these responses, some of these receptor studies should be repeated using a more sophisticated experimental design. From such experiments it is conceivable that the pharmacological profile of the tick SG will turn out to be somewhat different from what we have interpreted so far.

So far I have concentrated primarily on physiological aspects related directly to the tick itself, and more specifically to the secretion of saliva. But as stated earlier, most of our interest in ticks relates to the role of the SG in pathogen transmission. So let us now turn our attention to a few aspects of the role of tick SGs to pathogen transmission. Because of limited space, I cannot really do justice to this vast area, but I shall point the way to more comprehensive literature as I proceed.

Saliva-assisted transmission of tick-borne pathogens

A pathogen encounters several major barriers along its hazardous route from one host to the next. The first barrier is the vector's gut where the luminal contents could potentially be destructive. Then the gut epithelium presents a physical barrier that then must be penetrated. If that barrier is overcome, the vector's HL may contain numerous substances comprising the innate immune system that could be harmful to the pathogen (reviewed by Sonenshine and Hynes, 2008). Then there is the further physical barrier of the SG epithelium that has to be crossed, and the chemical content of the saliva that has to be endured, for the pathogen to reach the next host. However, it turns out that the saliva itself facilitates rather than attenuates the transmission of some pathogens.

Tick saliva contains numerous substances that enable the tick to maintain the flow of blood to the feeding lesion (Mans and Neitz, 2004; Steen et al., 2006; Mans et al., 2008a,b). At least some pathogens seem to exploit the pharmacological environment of tick saliva to enhance their own transmission. The first demonstration of this was by Jones et al. (1989), who demonstrated "saliva activated transmission" (subsequently renamed "saliva-assisted transmission"; SAT) of Thogoto virus by tick SGs. SAT can be demonstrated as follows. If Thogoto virus is syringe-inoculated into a guinea pig on which a number of nymphal *Rhipicephalus appendiculatus* are feeding, only about 6% of the nymphs subsequently

become infected with virus. But if the same dose of virus is inoculated within a SG extract prepared from uninfected ticks, there is typically a 10-fold increase in the number of nymphs that subsequently become infected (Nuttall, 1998). The SAT-factor responsible for this effect is a substance secreted into the saliva (Jones et al., 1992).

The phenomenon of SAT has been observed also for other tick-borne viruses and for bacteria such as *Borrelia* and *Franciscella* (reviewed by Nuttall and Labuda, 2008). Although the concept of SAT arose initially for several viruses, the precise identity of the SAT factor is not known for any virus, nor is it known whether the factor comprises one or more molecules. The SAT factor for *Borrelia burgdorferi* in the SG of *Ixodes scapularis* has been identified, however: Salp15 (Ramamoorthi et al., 2005). Salp15 is a 15 kD protein that binds to an outer surface protein of the spirochaete (OspC), where it offers protection against antibody-mediated killing. Salp15 inhibits CD4+ T-cell activation (Anguita et al., 2002), and RNAi of *salp15* markedly attenuates infectivity of the spirochaetes (Ramamoorthi et al., 2005). Unlike Salp15's direct mechanism of action on the spirochaete, however, the SAT factor of Thogoto virus acts on the host, not on the virus. Thus, the infectivity of the virus in mice was not enhanced by incubation with a tick SG extract (Jones et al., 1989). Furthermore, the Thogoto virus SAT-factor appears to act at the skin site specifically, rather than centrally, because if the virus and SG extract are injected at sites remote from each other, SAT is not observed (Jones et al., 1989). A fine review of all this and more is presented by Nuttall and Labuda (2008).

Mechanism of pathogen transfer across the SG epithelium

The techniques developed to study the physiology and pharmacology of salivary fluid secretion have proven particularly useful for examining several aspects of pathogen transmission. Two of those projects began with the following questions: (1) Can an arbovirus pass more-or-less directly from the HL across the epithelial cell layer of the SG, or must the virus infect the SG tissue, and undergo a period of development there before it can be transmitted successfully? (2) Is the physiology of the tick affected in any way when they are infected with an arbovirus?

Kaufman and Nuttall (1996) defined 'biological transmission' as the experimental condition in which a virus infection has been established in the tick. For this condition, nymphal *A. variegatum* were either fed on viraemic hamsters, or virus was inoculated into the

haemocoel of uninfected engorged nymphs. In either case, virus had time to disseminate to, and infect, the tissues of the tick, including the SGs. The resulting adults were then fed to varying degrees on guinea pigs, and injected with DA or PC to stimulate salivation. The saliva was then assayed for virus by means of a plaque assay. Kaufman and Nuttall (1996) defined 'mechanical transmission' as the condition in which virus was injected into the haemocoel of the partially fed adult at the same time as injecting the drug to stimulate fluid secretion. In this case, there was no time for a viral infection to be established before salivation was stimulated. Doses of virus were chosen to overlap and exceed the titres anticipated for the HL of naturally infected ticks. In a subsequent paper, 'biological' and 'mechanical' transmission were renamed to 'natural' and 'pericellular', respectively (Kaufman and Nuttall, 1999).

The frequency of virus-positive saliva in ticks injected with PC in the pericellular transfer experiment (29%) was significantly greater than the frequency of virus-positive saliva in ticks injected with DA (8%). Values for the very few naturally infected ticks that were available in that study were similar to the pericellular values (10% for DA and 17% for PC). We still do not know what causes this difference in efficacy between DA and PC.

In Fig. 6 (pericellular transfer), virus concentration in saliva is plotted as a function of virus concentration in the HL; panel A shows absolute virus titre in saliva and panel B shows virus titre expressed as a 'saliva-to-HL ratio' (S/H ratio). There was a significant negative correlation between S/H ratio and virus content of HL (Fig. 5B). The S/H ratio was between 0.03 and 0.07 when HL titre was in the range characteristic of naturally infected ticks (39,000 ± 13,000 PFU/ml). This range was similar to that of saliva from naturally infected ticks (S/H ratio of 0.008 to 0.119).

The relatively low frequency of positive saliva samples in the pericellular and natural transfer experiments (10–30%) could be due to the relatively small volumes of saliva available from each tick (around 10 µl per sample). Each saliva sample is only a single snapshot of a complex process, so it is easy to imagine that many such individual samples may not contain virus. This was supported by findings on a population of engorged ticks from which we were able to collect serial samples of saliva (up to five serial samples of 4–16 µl per

sample), and measure each successive sample for virus titre. Virus titre varied from 0 PFU/ml to 21,000 PFU/ml in any one series, with adjacent samples being randomly high or low (Kaufman and Nuttall, 1996).

The foregoing suggests that virus is secreted into the saliva in a highly idiosyncratic way, at least under these artificial conditions (stimulating salivation by exogenous drugs). However, one might expect intuitively that the amount of virus secreted would be correlated with the absolute volume of saliva secreted. At least this was the argument I used at the beginning of this review for explaining why ticks are particularly effective transmitters of pathogens. So Kaufman and Nuttall (2000) hypothesized that secretion of virus might be enhanced by drugs known to stimulate salivary fluid secretion. Using *Rh. appendiculatus*, we bathed isolated SGs from infected ticks in high concentrations of DA, ergometrine, GABA, or DA plus GABA. We also tested the effect of prostaglandin E_2 on virus secretion, because PGE_2 was postulated by Quian et al. (1997, 1998) to play an important role in the signal transduction pathway of salivary fluid secretion. At least some pathogens are believed to enter cells by endocytosis at the baso-lateral surface and exit these cells by exocytosis at the apical surface (Munderloh and Kurtti, 1995), and prostaglandins are known to be mediators of endo/exocytosis on tick SGs via their effect on mobilizing intracellular Ca^{2+} (Yuan et al., 2000). However, the amount of virus secreted by infected SGs in vitro, subjected to any of these drugs known to stimulate or modulate fluid secretion, was not significantly different from the amount of virus secreted by SGs exposed to bathing medium alone (Kaufman and Nuttall, 2000). These results suggest that virus release from infected SGs is controlled at least somewhat independently of fluid secretion.

Does an arbovirus infection influence the physiology of the tick?

Pathogens are usually assumed to have little if any effect on the behaviour of their vectors, but often this may be because one has to guess at what behaviours might be affected, and then measure these behaviours under defined conditions. It is reasonable to hypothesize that a virus infecting the SGs might have an effect on rate of fluid secretion. Two reasonable hypotheses would be (1) viral infection might inhibit fluid secretion because of cellular damage to the SG, or (2) viral infection might enhance salivation, because that would be advantageous to the virus. There is abundant precedence for the second view: many

parasites manipulate the behavioral physiology of their hosts or vectors in such a way as to promote the likelihood of their transmission (Moore, 1984; Libersat et al., 2009).

Kaufman and Nuttall (1996) collected saliva following injection of either DA or PC into either uninfected or infected ticks. The saliva volume from the naturally infected ticks injected with PC was 27% lower than from the uninfected ticks, and 31% lower than from the pericellularly infected ticks. For DA the trend was similar, but the 10% difference between infected and non-infected ticks was not statistically significant (Fig. 7). The fact that simple inoculation of virus (pericellular) did not inhibit salivary fluid secretion indicates that reduction in fluid secretion was a consequence of cellular infection and suggests that the virus has a deleterious effect on the fluid secretory process. But on further reflection, there was a more intriguing possibility. It is possible that fluid secretion in the attached tick was augmented by the virus infection, and if so, the HL volume of infected ticks removed from the host could have been lower than that in the controls. Hence, the amount of saliva collected following drug injection into detached infected ticks would have been less than from the controls. Kaufman, Bowman and Nuttall (2001) measured HL volume in control and infected ticks at various stages of the feeding cycle, and found that they were virtually identical in the two groups (23-24% bw). They also tested the rate of fluid secretion in vitro in the two groups, because under an in vitro experimental protocol, the volume of fluid bathing the gland would not be a limiting factor for rate of fluid secretion. As feeding progresses, the wet weight of the SGs increased significantly and then reached a plateau, as expected for feeding ticks, but there was no apparent difference in tissue weight throughout the feeding cycle between control and infected SGs (Fig. 8A). Throughout the slow phase of engorgement, the virus-infected glands secreted at approximately 70-80% the rate of the controls — very similar to the difference originally observed by Kaufman and Nuttall (1996) *in vivo* (73%). But for large and engorged ticks, there was an apparent 18% increase in fluid transport in infected SGs over the control, although this difference was not statistically significant. This difference between the partially fed and engorged ticks is puzzling. We also do not know where within the cells the virus is acting. Some data suggested that it occurs at a point downstream from adenylate cyclase activation, although a more proximal site could not be ruled out entirely (Kaufman, Bowman and Nuttall, 2001). Statistics aside, what might be the biological significance of the virus inhibiting secretion early in feeding and possibly enhancing it later? A clue might arise once we have better data on the dynamics of virus secretion throughout a

normal feeding period. Although transmission of virus can *begin* within 24 hours of attachment, and continues throughout the feeding cycle (Davies, 1988), nobody has yet established *how much* virus is secreted at various points in the feeding cycle. Until we have the appropriate data, it is reasonable to imagine that most virus is secreted during the rapid phase of engorgement, and if so, perhaps the virus really can manipulate the behaviour of the vector to its advantage.

General Conclusions

It may not have escaped the reader that much of the literature referred to in this review is 20 or more years old. Since molecular approaches to the physiology of vectors have now become part of our normal repertoire, it may sometimes be forgotten that classical physiological techniques still remain appropriate for answering many fundamental questions. Although we now have some foundation of basic knowledge in tick physiology, much superstructure remains to be built, and classical in vivo and in vitro techniques still have important roles to play in that enterprise.

The wide spectrum of biologists interested in ticks and the diseases they transmit form a very small pond in the world of biology, and the physiologists among us occupy only a small region of that pond. Much of our work does indeed contribute to the solution of medical-veterinary problems. One prominent example is the considerable progress we have made in understanding tick-host interactions, an understanding that has led to the prospect of one day weaning ourselves from a heavy reliance on environmentally harmful acaricides to control tick populations that plague us, our livestock and our domestic animals. But to date there are only two commercially available anti-tick vaccines (TickGARD® - later TickGARD Plus® - and Gavac® - later Gavac Plus®; Willadsen, 2008), both based on the same antigen (Bm 86), and both authorized for use on only one tick species (*Rh. microplus*). Nevertheless, there is every reason to hope that before too long we may have other vaccines, based on multiple antigens, and effective for multiple tick species (Brossard and Wikel, 2008; Willadsen, 2008). However, without minimizing the importance of tick research applied to human welfare, we should also remember that ticks are not merely vectors that must be kept under control, and their SG proteins are not merely sources for new pharmaceutical agents (Anderson and Valenzuela, 2008), as intriguing as that possibility is. Perhaps I have shown here that ticks are most

fascinating creatures in their own right, and that they have a lot to teach us about general biology.

Acknowledgements

I am most grateful to the organizers of the X International Jena Symposium on Tick-Borne Diseases for providing me with travel expenses to the Symposium. Research in my laboratory has been generously funded in an ongoing manner by NSERC Canada. The virus research presented here was carried out in collaboration with Prof. Patricia Nuttall over several visits, and over a number of years, at the former NERC Institute of Virology and Environmental Microbiology (now CEH), Oxford. These visits were supported generously by the following granting agencies: The Burroughs-Wellcome Fund (Travel Grant), The Royal Society (Guest Fellowship), NSERC (International Collaborative Research Grant), The Alberta Heritage Foundation for Medical Research (AHFMR; Visiting Scientist from Alberta Award), and The British Council in Canada (Academic Travel Grant). Finally, I wish to acknowledge the most valuable feedback I received from the three anonymous reviewers.

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Figure Legends

Figure 1. Diagram of the coxal organ of the argasid tick, *Ornithodoros moubata*. **cg**: coxal gland: glandular tissue associated with the terminal portion of the coxal tubule. Its function is unknown, although it has been proposed to secrete a known argasid tick sex pheromone (Schlein and Gunders, 1981). Based on a variety of circumstantial evidence, Binnington (1975) proposed that a similar (perhaps homologous) gland in ixodid ticks may perform some function during the moulting process. **d:** terminal duct portion of coxal tubule. **co:** orifice of coxal organ. The orifice appears externally between the first and second coxae. **fm:** filtration membrane. Numerous fine muscles (not shown) anchor the membrane to the internal body wall. **ct:** coxal tubule, where reabsorption from the ultrafiltrate of HL occurs. **m:** a muscle that anchors the coxal organ to the internal body wall. Figure from Kaufman, Kaufman and Phillips (1981) with kind permission from the Company of Biologists.

Figure 2. (A) Ultrastructure of the filtration membrane of the coxal organ of *Ornithodoros moubata*. The basic arrangement of podocytes with pedicel processes aligned along a basal lamina is very similar to what one would see in a vertebrate glomerular nephron. Lacking, however, is an underlying capillary endothelium because ticks have an open circulatory system, unlike vertebrates. The arrow points to a pedicel (Pe). The cell body of a podocyte (Po_1) is also indicated. Figure modified from Hecker et al., 1969, and reproduced with kind permission of Elsevier. (B) Ultrastructure of the coxal tubule of *Ornithodoros moubata*. Major components are labeled. Figure reproduced from Kaufman (1971) with kind permission of Dr. S.E. Jacobs-Kaufman.

Figure 3. Salivary fluid secretion alone accounts for HL volume regulation. Various solutions containing ^{14}C-PEG were injected (25 µl/100 mg bw) into the HL. The volume of saliva secreted over the subsequent hour or so was measured, and the subsequent HL volume was calculated from the dilution of ^{14}C-PEG in the HL space. Injected solutions were, (open circles): 1.2% NaCl injected within 1 hour post removal from the host. (Open triangles): 1.2% NaCl injected 40 hours post removal from the host. (Closed circles): 1.2% NaCl injected 40-72 hours post removal from the host, followed 3 hours later with 53 nM DA (1 µl/100 mg bw) to induce salivation. (Closed triangles): 11.2% sucrose injected within 1 hour post removal from the host. (Open square): Normal HL volume of

partially fed ticks as measured by ^{14}C-PEG. Figure modified from Kaufman, Aeschlimann and Diehl (1980) and reproduced with kind permission of the American Physiological Society.

Figure 4: Diagrammatic summary of the intracellular signaling pathways mediating salivary gland secretory processes in ixodid ticks. The DA neurotransmitter, acting via its G-protein (Gs)-coupled receptor, causes the release of cAMP into the cytosol. Numerous intracellular proteins are subsequently phosphorylated by cAMP-activated protein kinase. Prominent among these proteins is postulated to be a family of aquaporins (AQP), or water channel proteins, that become inserted into the cell membrane, where they promote the passage of fluid across specific cells of the SG epithelium. DA also stimulates the uptake of extracellular Ca^{2+} into the fluid secretory cell via voltage-gated calcium channels. Ca^{2+} stimulates a cytosolic phospholipase A_2 ($cPLA_2$) which liberates free arachidonic acid (AA). AA is converted to a number of prostaglandins, including PGE_2, via cyclooxygenase (COX). PGE_2 is secreted into the saliva where it is hypothesized to have antihaemostatic, vasodilatory, immunosuppressive and anti-inflammatory activities. PGE_2 also has a paracrine or autocrine effect on the SG, where it activates phospholipase C (PLC) to release the intracellular messenger, inositol triphosphate (IP_3) and diacyl glycerol (DAG). IP_3 in turn stimulates the release of intracellular calcium (Ca^{2+}_i) from the endoplasmic reticulum (ER) into the cytosol. Ca^{2+}_I mediates exocytosis of various secretory vesicles, including those containing anticoagulant proteins. A more detailed description of this intracellular signaling cascade is provided by Bowman et al. (2008). Figure reproduced from Sauer et al. (2000), with kind permission from Elsevier.

Figure 5. Model diagram summarizing the known pharmacology of tick salivary fluid secretion by 1989. The main salivary secretory nerve is dopaminergic, stimulating fluid secretion via adenylate cyclase and calcium ion intracellular signaling pathways. The interactions of the intracellular pathways shown here are much oversimplified. See Fig. 4 for a more complete exposition. Two sensory systems impinge on the secretomotor nerve: a cholinergic pathway, the physiological significance of which is unknown, and a non-cholingergic pathway (neurotransmitter unknown) that monitors HL volume. The ergot alkaloid pathway also stimulates fluid secretion, but the “??” indicates that the intracellular signaling pathway for this system is unknown. Also unknown is the natural ligand for this pathway (“?”), as well as the physiological conditions that lead to

activation of this pathway. The GABA-receptor is not linked directly to fluid secretion (i.e., GABA stimulates very little fluid secretion on its own), but GABA potentiates both the DA-responses and ergot alkaloid responses, the mechanism of potentiation being unknown. Figure reproduced from Kaufman (1989) with kind permission of Elsevier.

Figure 6. Saliva virus titre as a function of HL virus titre (pericellular transfer) in partially fed *A. variegatum* females. Virus titres were measured by plaque assay (see Kaufman and Nuttall, 1996). (A) Absolute virus titre (PFU/ml saliva). Although saliva virus titre was highly variable, the slope of the regression curve was not statistically significantly different from 0. (B) saliva-to-haemolymph (S/H) ratio. The S/H ratio of virus titre declined significantly with increasing HL titre. The shaded bars indicate the S/H ratio when the HL titre was in the range of normally infected ticks (39,000 ± 13,000 PFU/ml). Figure modified from Kaufman and Nuttall, 1996 and reproduced with kind permission of Elsevier.

Figure 7. Saliva volume secreted as a function of virus exposure in *A. variegatum*. The three conditions shown for each drug (pilocarpine and dopamine) are: naturally infected, pericellular transfer and uninfected. All ticks (females) were partially fed. Figure modified from Kaufman and Nuttall, 1996 and reproduced with kind permission of Elsevier.

Figure 8. The effect of Thogoto virus on (A) SG wet weight and (B) salivary fluid secretory competence in naturally infected ticks (*Rh. appendiculatus*) at various stages of the feeding cycle. Ticks were pooled into the four weight ranges indicated. Sample size is indicated near each symbol. Virus infection had no effect on SG wet weight, but significantly inhibited fluid secretion in the small partially fed ticks (<200 mg). Virus had no statistically significant effect on fluid secretion in large partially fed ticks (> 200 mg). Figure modified from Kaufman, Bowman and Nuttall, 2001 and reproduced with kind permission of Springer Science and Business Media.

Figure

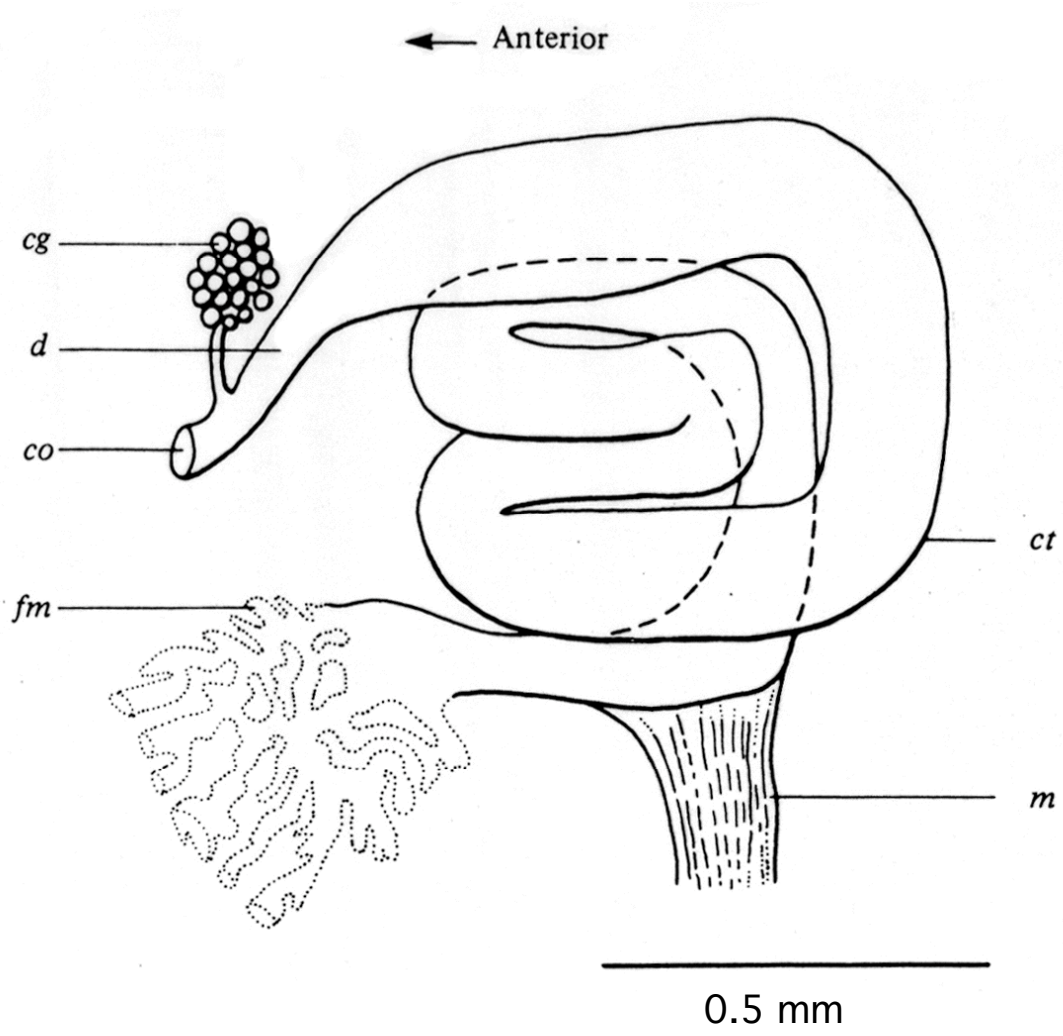


Figure 1

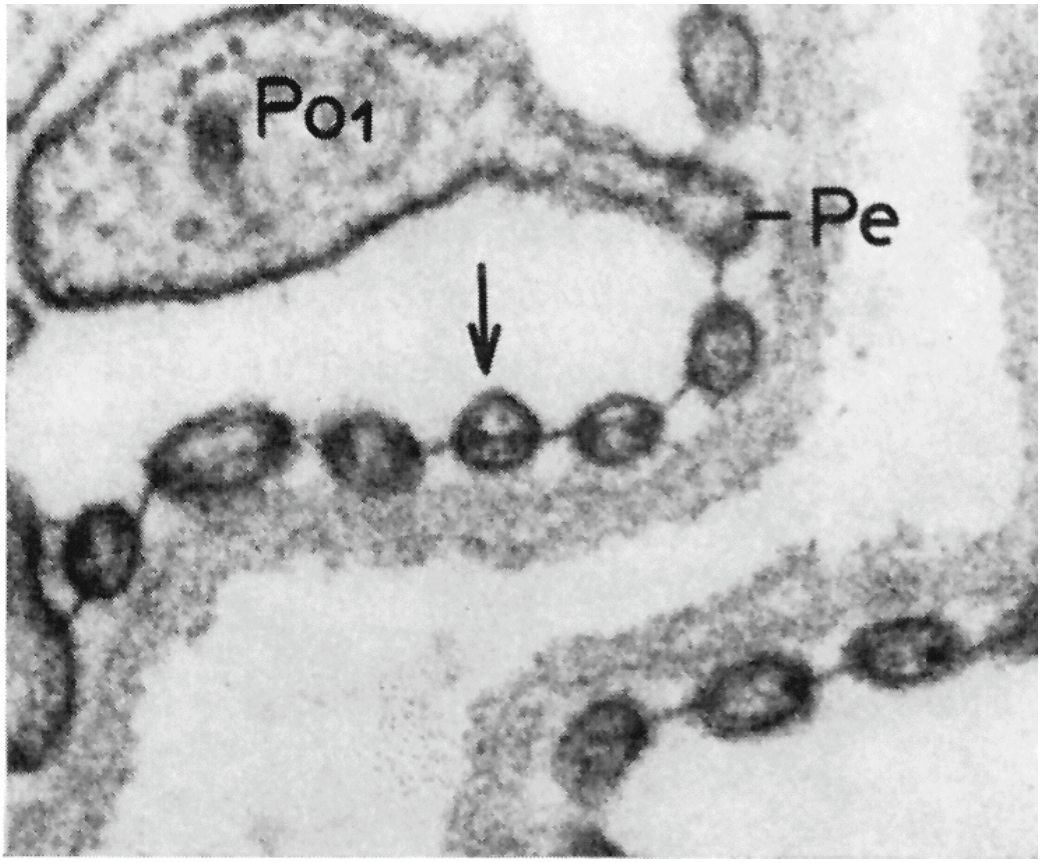


Figure 2A

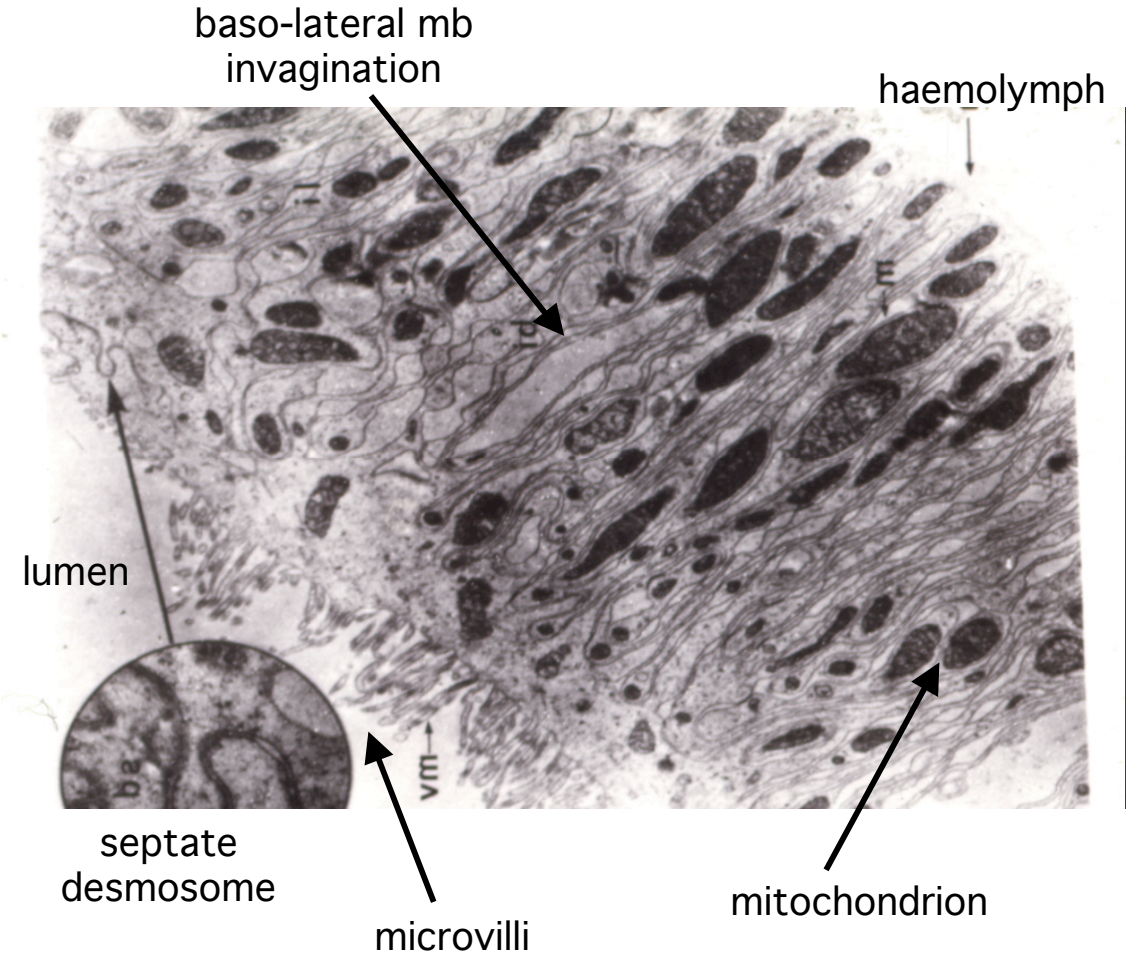

baso-lateral mb
invagination
haemolymph
lumen
septate
desmosome
microvilli
mitochondrion

Figure 2B

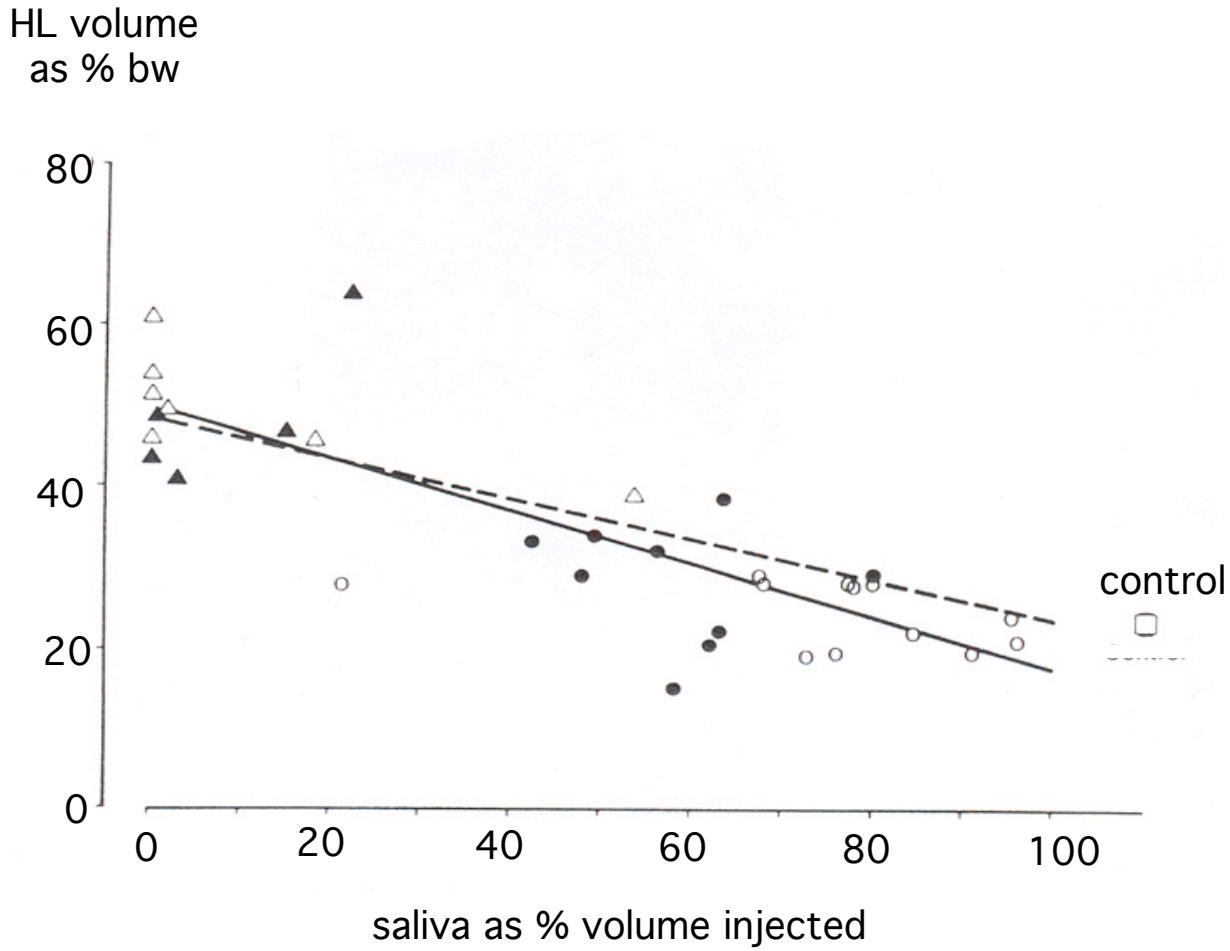

HL volume
as % bw
80
60
40
20
0
0
20
40
60
80
100
saliva as % volume injected
control

Figure 3.

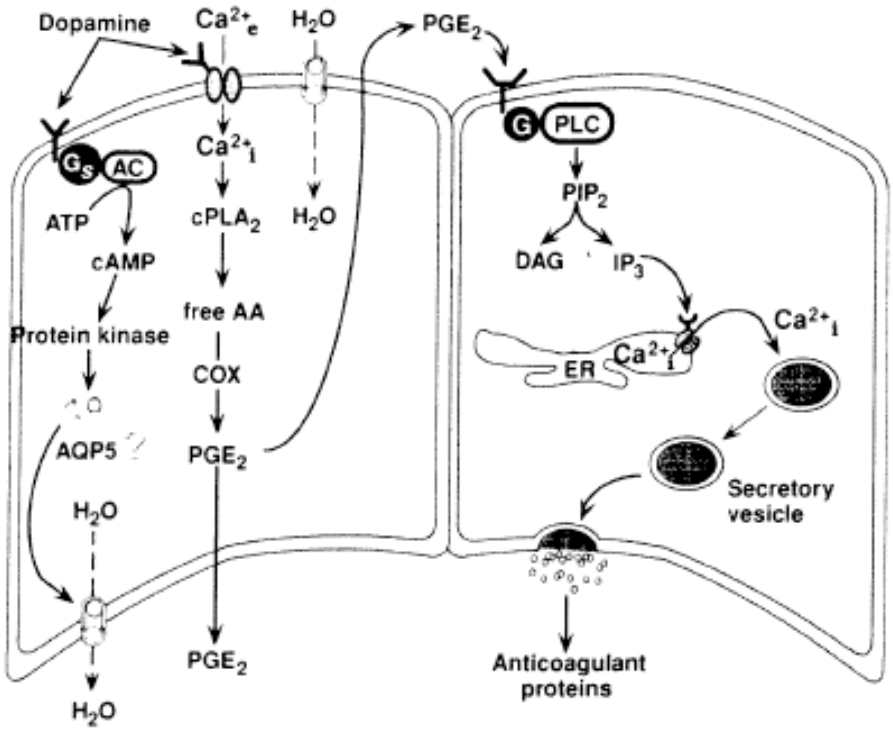

Dopamine
Ca^{2+}_e
H_2O
PGE_2
G_s
AC
ATP
cAMP
Protein kinase
AQP5
H_2O
H_2O
Ca^{2+}_i
$cPLA_2$
free AA
COX
PGE_2
PGE_2
H_2O
G
PLC
PIP_2
DAG
IP_3
ER
Ca^{2+}_i
Ca^{2+}_i
Secretory vesicle
Anticoagulant proteins

Figure 4.

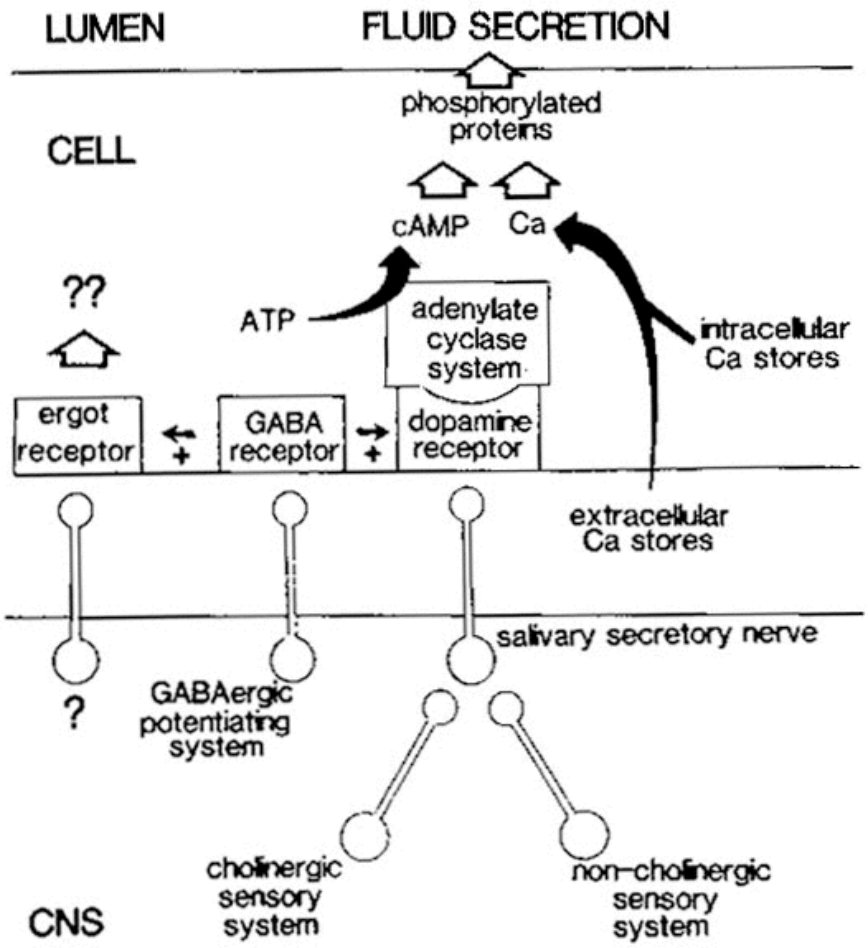

LUMEN
FLUID SECRETION
CELL
phosphorylated proteins
cAMP
Ca
??
ATP
adenylate cyclase system
intracellular Ca stores
ergot receptor
GABA receptor
dopamine receptor
extracellular Ca stores
salivary secretory nerve
?
GABAergic potentiating system
cholinergic sensory system
non-cholinergic sensory system
CNS

Figure 5.

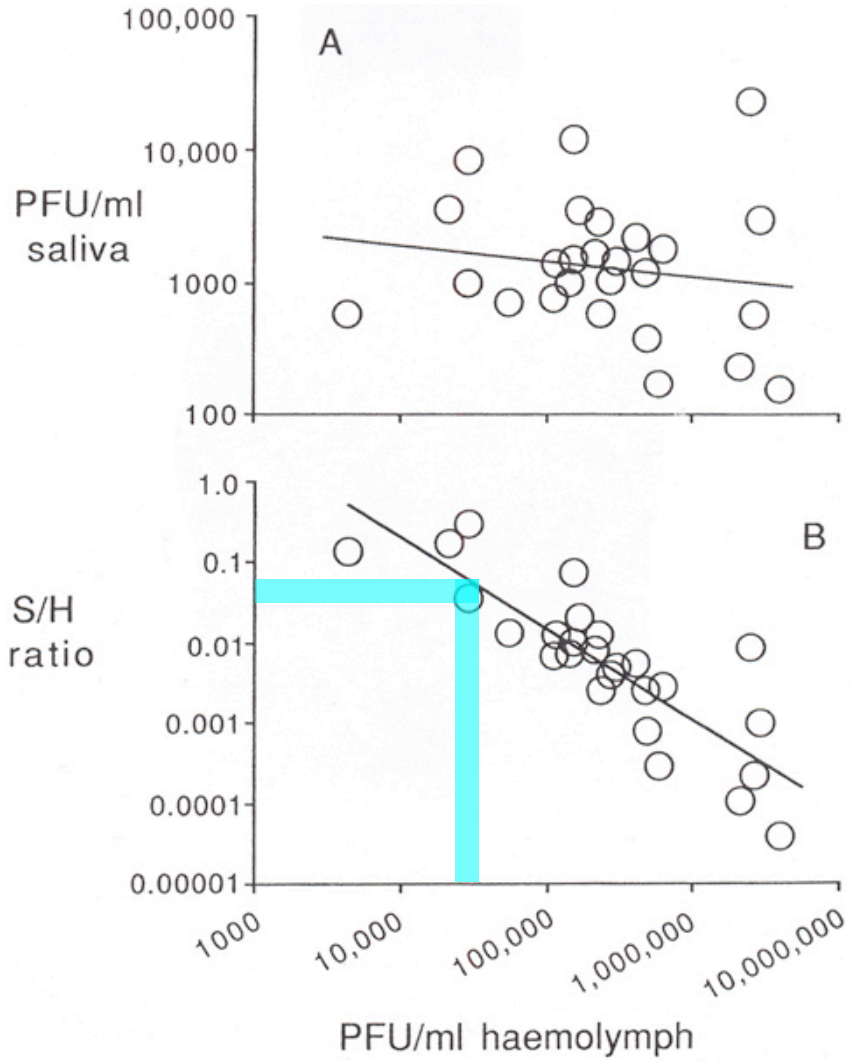

100,000
10,000
1000
100
A
PFU/ml
saliva
1.0
0.1
0.01
0.001
0.0001
0.00001
B
S/H
ratio
1000
10,000
100,000
1,000,000
10,000,000
PFU/ml haemolymph

Figure 6

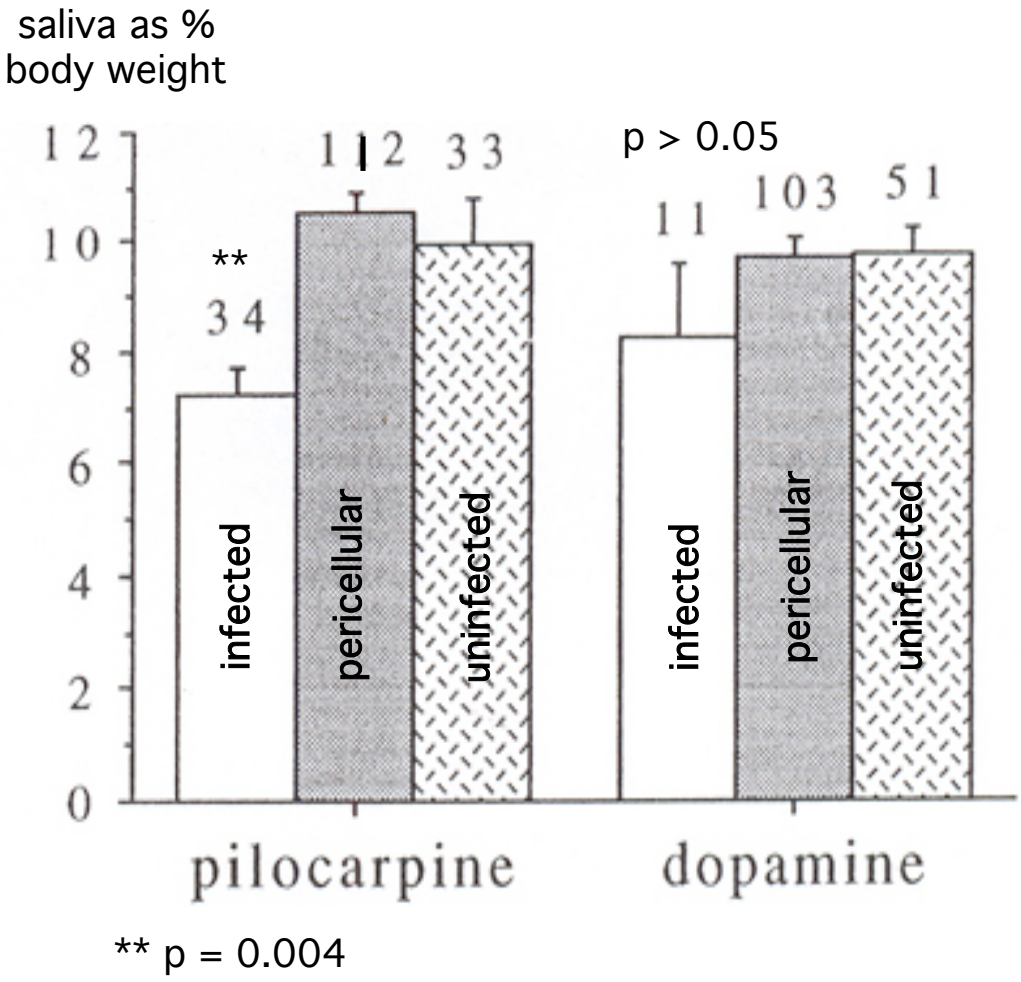
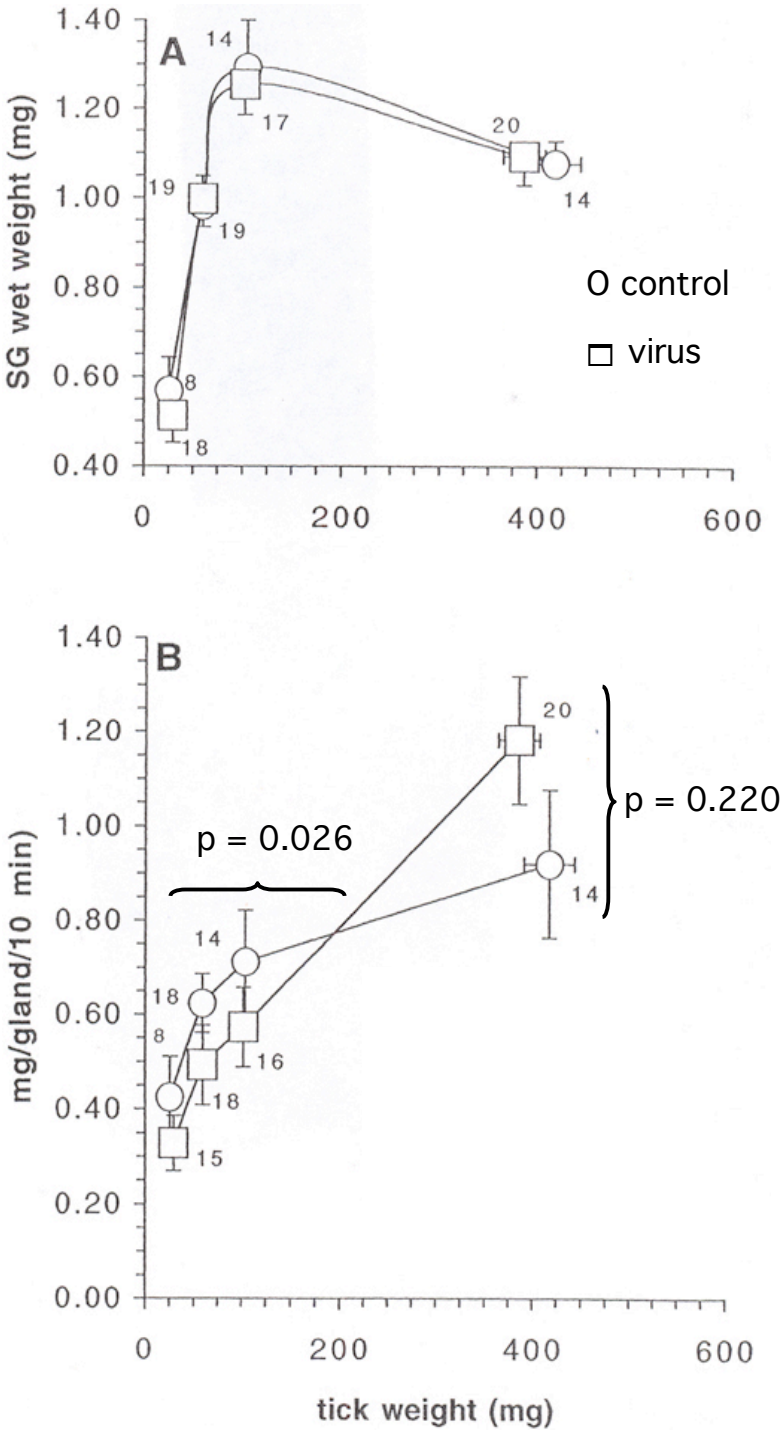

saliva as %
body weight
12
10
8
6
4
2
0
**
34
112
33
p > 0.05
11
103
51
infected
pericellular
uninfected
infected
pericellular
uninfected
pilocarpine
dopamine
** p = 0.004

Figure 7.



tick weight ranges

< 40 mg

40-80 mg

80-200 mg

> 200 mg

Figure 8.