

Feeding electrogram studies on the African cattle brown ear tick *Rhipicephalus appendiculatus*: evidence for an antifeeding effect of tick resistant serum

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Abstract. Feeding behaviour of partially engorged *Rhipicephalus appendiculatus* (Neumann) (Acari: Ixodidae) on rabbit serum held in capillary tubes and placed over the tick mouthparts was studied using the feeding electrogram technique with simultaneous macro video photography. Correlation of electrical events with fluid movement in the vicinity of the tick's mouthparts and the capillary meniscus, permitted the characterization of an orderly sequence of signals, termed the 'Feeding Complex', associated with highest weight gains. This complex consisted of a 3–8 Hz fast-sucking waveform typically lasting 4–5 min, a sharp drop in potential at salivation, and rest lasting 1 or 2 min where no waveform or fluid movements occur. Very high impedance recordings from within the tick capitulum indicate that the fast-sucking waveform coincides with bursts of potentials corresponding to contraction of the pharyngeal dilator muscles, whereas during rest a tonic series of spikes signifies that the floor of the salivarium is actively lowered. Feeding electrograms of ticks fed on serum from tick-resistant rabbits showed significantly fewer feeding complexes. The weight gains achieved by these ticks were reduced correspondingly. This suggests that some of the humoral effectors of immunity have an antifeedant effect on this unusual parasite of rabbits.

Key words. Feeding electrogram, ticks, *in vitro* feeding, *Rhipicephalus appendiculatus*, tick resistance, ectoparasite, antifeedant.

Introduction

In vitro feeding of ticks is a valuable tool in detailed studies of feeding behaviour, and allows for quantifiable changes in the tick's environment which could not be achieved on the host animal. This study investigates the tick's response to properties of dietary media forced onto its mouthparts. In the past, two main artificial feeding systems have been used to study hard tick feeding: the capillary system consisting of a drawn capillary tube diet-reservoir placed over the ticks' mouthparts (Chabaud, 1950), and various artificial membranes through which the ticks feed on a pool of diet (e.g. Stone *et al.*, 1983; Waladde *et al.*, 1979).

As simple indices of tick *in vitro* feeding performance, Joyner & Purnell (1968) measured the decrease in feeding capillary volume, Willadsen *et al.* (1984) determined tick weight gains, and Waladde *et al.* (1979) made scintillation counts on the tissue of ticks which had fed on radiolabelled diets. Feeding electrogram techniques, originally devised for recordings from phytophagous insects (e.g. McLean & Kinsey, 1964; Tjallingii, 1978) but also widely used in behavioural studies on haematophages (e.g. Kashin & Wakeley, 1965; Smith & Friend, 1970) have also been successfully applied to tick feeding behaviour (Gregson, 1967; Tatchell *et al.*, 1972; Waladde *et al.*, 1979; Paine *et al.*, 1983). In this study we describe the feeding electrogram of the African cattle brown ear tick, *Rhipicephalus appendiculatus* (Neumann) (Acari: Ixodidae), feeding on glass capillaries filled with rabbit serum. Components of the feeding electrogram, observed to occur when ticks take up most weight, were correlated with clearly defined behaviours such as sucking and salivation by video-assisted monitoring of the tick's activities.

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Mammals acquire a certain degree of immunity to ticks via previous infestations (Wikel & Allen, 1982). This 'naturally acquired immunity' is at least in part due to humoral factors (e.g. Trager, 1939; Brossard & Girardin, 1986; Rubaire-Akiki & Mutinga, 1980). In rabbits, this resistance is illustrated by dramatic reductions in female engorgement weights with successive tick-infestations. In the present study, *R. appendiculatus* engorgement weights declined from 300–500 mg for the first infestation to under 70 mg in the third infestation. Components of the feeding electrogram correlated with successful feeding are employed here to test if serum of rabbits, which had acquired this natural resistance to *R. appendiculatus*, also affected feeding by these ticks *in vitro*.

Materials and Methods

Supply and maintenance of ticks. Three- to 6-month-old *R. appendiculatus* adults, in a 1:1 male:female ratio, were supplied at regular intervals from the culture maintained at The International Centre for Insect Physiology and Ecology (I.C.I.P.E.), Nairobi, Kenya. Ticks were stored in darkness, at r.h. > 95% and 27°C. Prior to *in vitro* feeding, ticks were allowed to attach to and feed from the ears of 2.5 kg, male, New Zealand white strain, laboratory rabbits. Twenty-five pairs of male and female ticks per ear were released into cloth bags taped so as to enclose the ears of the rabbits. Females attached within 24 h of release on the host and were allowed to feed for a total of 5–7 days. These partially engorged females, judged by their size to lie in the 35–70 mg weight range, were carefully removed with forceps from the rabbit's ears for use in experiments. All males, and any females with damaged mouthparts or which exceeded the required weight range were discarded.

Preparation of test sera. Blood collections were routinely made between 14.00 and 16.00 hours to minimize possible effects of the rabbit's circadian rhythm. 8–15 ml blood samples were collected from the ear marginal vein of four, 2.5 kg male rabbits, 1 or 2 weeks before the first infestation and 1 week after the second infestation. Centrifugation (4000 rpm, 15 min) yielded approximately half this volume of 'naive' and 'pluri-infested' sera respectively.

In vitro feeding tests. Two systems for the artificial nutrition of hard ticks were considered, the capillary feeding system based on Chabaud (1950) and an artificial membrane system. Due to the very limited volumes of test sera available the former proved more appropriate to the work described below.

Ten centimetre lengths of 2 mm o.d. and 1.2 mm i.d. borosilicate glass tubes (Hilgenberg, GmbH) were drawn by an electrode puller (Ealing) to give capillary tips with even taper and strong walls. The tips were reduced on fine abrasive paper to give a 300–350 µm opening, which could be slipped easily under the palps and over the tick's hypostome and chelicerae. The capillary tube was filled with serum and placed over the mouthparts of a tick, mounted ventral side up on a microscope slide and held by a piece

of double-sided sticky tape. The free end of the capillary tube, held in 'Plasticine', was inclined at an angle of about 35° to the horizontal.

Feeding electrograms. A 5 cm-long chloridized silver wire electrode was inserted into the distal end of the feeding capillary, and connected to the probe of an 'Electrical Penetration Graph' amplifier (Syntech, Hilversum), built to the design of Tjallingii (1978). The amplifier was a non-inverting, direct current type with an input impedance of 10^9 Ohms and a gain of 50. The indifferent connection to the tick was made with a chloridized silver wire implanted in the coxa of limb iv. With this amplifier, a small potential ranging from + to – 160 mV was applied across the preparation. The size of the potential was adjusted at the start of a recording to counter 'back-potentials' from the electrode contacts, and to set an appropriate output signal for display on a storage screen cathode ray oscilloscope and chart recorder (frequency response, 0–75 Hz). Slow drift and slight attenuation of the signal which occurred over a number of hours was attributed to deposition of haemolymph components on the electrode embedded in the tick.

Macro-video filming with two cameras was employed to correlate feeding behaviour with the feeding electrogram of *R. appendiculatus*. A colour camera (Cannon Ci 20) mounted on a Wild-Leitz M5 stereo microscope ($\times 50$) allowed the activities of the tick mouthparts, transilluminated with a cold light source, to be viewed on a colour T.V. monitor (Sony Trinitron). The uptake of serum and the release of saliva by the tick could be seen clearly by judicious adjustment of lighting. The other video camera (Canon Ci 20PR), equipped with a Nikon $f = 100$ mm/2.8 macro-lens mounted on extension rings, permitted changes in position of the serum-meniscus inside the capillary tube to be viewed at a magnification of about $50\times$ on a 9 inch monochrome TV monitor. As it was not possible to merge the outputs of the two cameras with the equipment available, recordings were made alternately on a VHS format video cassette recorder (Panasonic) with the signal from one camera or the other. Volume changes associated with sucking and salivation phases of the tick's behaviour (described below) could easily be calculated with reference to the internal diameter of the capillary tube. The relatively low frequency feeding electrogram trace was combined with a high frequency 'audio' carrier signal so that it could be recorded on one of the sound tracks of the video cassette recorder. On replaying the video recordings, precise correlations of electrical events with the activities around the mouthparts or changes in the meniscus level were achieved.

It was possible to make detailed recordings of more rapid electrical potentials in the tick's capitulum, associated with sucking and salivation phases of the feeding electrogram, using a general purpose single-ended configuration electrophysiological amplifier (Syntech, Hilversum, model UN03; probe input-impedance 10^{15} Ω, Gain $100\times$, adjustable filters on the output: D.C. output $f_{c(\text{low pass})}$ 50–2000 Hz; A.C. output $f_{c(\text{high pass})}$ 20–1000 Hz, $f_{c(\text{low pass})}$ 50–2000 Hz). The recording electrode connection to the

amplifier was a 0.15 M NaCl-filled glass micro-electrode inserted into the back of the capitulum through a window cut in the tick's alloscutum. The silver wire immersed in the serum-filled capillary provided the indifferent contact for this amplifier.

Experimental procedure for testing sera. From preliminary tests it was deduced that a 3 h period of feeding on any particular serum was required to reflect accurately the tick's feeding behaviour. Comparison of feeding electrogram responses for naive versus pluri-infested sera was made in 9 h bracketing experiments in which the tick was fed naive serum for the first and last 3 h of the experiment, and on the pluri-infested serum during the middle 3 h. When the recording was interrupted to change or, as at the end, to remove the feeding capillary, the tick was weighed together with the microscope slide on which it was mounted and the reference electrode still in place. After the tick had been removed, the slide and the electrode were again weighed to determine the true weight of the tick. Weight gains throughout the experiment were all expressed as a percentage of the tick's initial weight.

The feeding electrograms were analysed with reference to the five 'pattern categories' described in the Results section, where the duration of each of these patterns was expressed as a percentage of the 3 h recording period. For a specific, recurring sequence of the three most clearly defined patterns, which make up the 'feeding complex' (below), more detailed analysis of the average duration of fast sucking and rest patterns, and the frequency of salivation events was undertaken. Because the 'normal' condition for ticks in the selected weight range was to feed rapidly *in vivo*, only those ticks feeding rapidly and therefore displaying this feeding complex were selected for recordings. Despite this, some ticks which continued to feed — as indicated by weight gains, had still to be rejected due to fading or drifting of the signal, injury to the mouthparts during changes of capillary or death in the course of 9 h experiments. Hence the successive reductions in number of replicates cited in Fig. 3(a–c). In experiments examining the tick's response to different sera, there inevitably is a 'bias' towards ticks which will readily accept this incomplete food substrate, *in vitro*, in place of whole blood taken up by these ticks feeding *in vivo*.

The results from all 'sets' of sera (i.e. naive and pluri-infested sera from four rabbits) were pooled for statistical analysis. Percentages of 3 h recordings showing the different pattern categories were analysed with Student-Newman-Keuls multiple range procedure, after log transformation of the data. This test was also applied to the weight gain data.

Results

Feeding electrograms

Despite considerable variability in features of the feeding electrogram between randomly selected individuals of similar weight which fed on control serum, it was still possible to identify five distinct categories. Signal 'level' (the vertical displacement of the trace on the chart-recorder),

signal frequency (the stroke rate of modulations around the level) and signal amplitude (the relative size of these modulations), were the main criteria for trace analysis. Typical examples of the five pattern categories, designated according to the following criteria, are illustrated in Fig. 1.

1. A 3–8 Hz waveform, occurring for 1–10 min typically with amplitudes of between 1 and 5 mV (Fig. 1a, Fsk).

2. Sudden drops in the recorded voltage level (of 5–20 mV) lasting for several seconds during which no waveform is observed (Fig. 1a, Sal).

3. Periods ranging from less than 1 min up to 5 min in length, immediately following pattern 2 and at a voltage level between those of 1 and 2. No waveform is observed (Fig. 1a, Rest).

4. A waveform of less than 1 mV with a frequency under 1 Hz, lasting for periods of a few minutes to several hours (Fig. 1b, Ssk).

5. Very mixed waveform in both signal frequencies and amplitudes, with no clear repetition of sequences. Frequently there is an underlying signal of pattern 4 (above) with periods of irregular higher frequency signals (over 20 Hz) of larger amplitude (1–10 mV) superimposed on it (Fig. 1c, Sk/Sal).

Correlations with feeding behaviour

When the type 1 pattern was recorded, rapid streaming of serum into the tick was observed. The flow followed a pulse-like motion, each 'pulse' corresponding to a wave unit recorded on the paper trace (Fig. 1a, insert). Regular contraction and relaxation of muscles around the tick pharynx, revealed by strong transillumination of the whole capitulum, coincided in frequency with the observed waveform. The fastest decreases in serum meniscus level in the capillary were also observed to coincide with this pattern and it will therefore be referred to as 'fast sucking' (Fsk).

Abrupt cessation of Fsk followed by the rapid voltage drop marking the onset of pattern 2 was always accompanied by a single 'burst-like' release of a large volume (typically, $0.12 \pm 0.08 \mu\text{l}$) of a clear fluid from the tick into the capillary tube. In contrast to the typical coloration of the serum, the fluid observed flowing out of the tick was colourless, indicative of salivation (Sal). The absence of any regular electrical activity when pattern 3 was recorded, corresponded to a period of no clearly directed flow into or out of the tick. It therefore appears to indicate a 'rest' period. Any fluid movements which did occur in the capillary consisted of 'swirling' around the mouthparts following the burst-like ejection of saliva by the tick.

Observations on the capillary volume where pattern 4 signals were recorded showed a slow sustained decrease in the serum meniscus level in the feeding capillary. The pattern will therefore be referred to as 'slow sucking' (Ssk in Fig. 1b). Flow rates were too low to be clearly visible at the mouthparts.

When pattern 5 occurred, slow fluid movements into and out of the capillary were observed. Occasionally, no clear movement could be discerned despite a 'trembling'

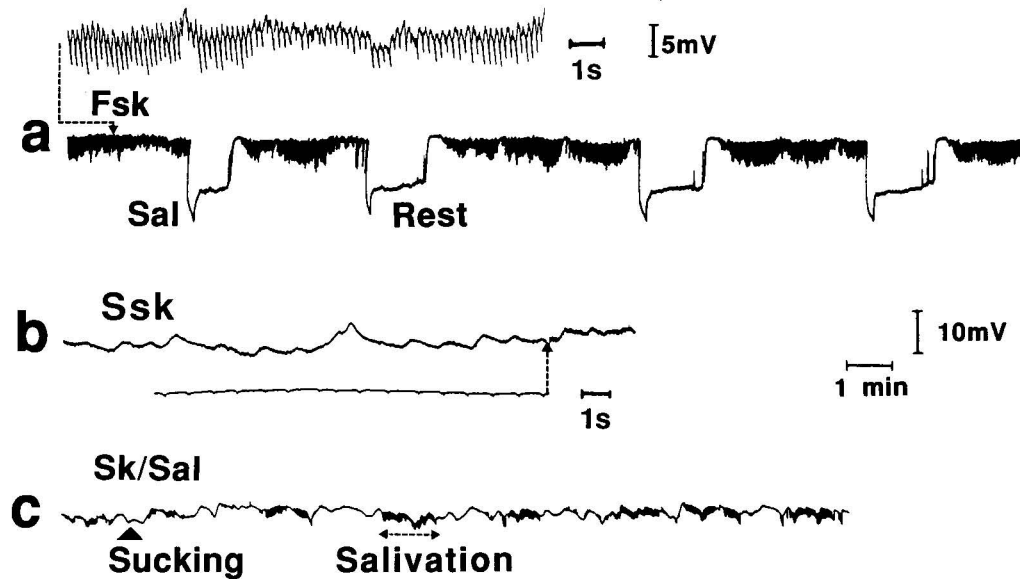


Fig. 1. Feeding electrogram waveform patterns: (a) 'feeding complex' sequence made up of 3–4 Hz strokes signifying fast sucking (Fsk), a drop in trace 'level' signalling release of saliva (Sal) followed by rest. Insert shows detail of fast sucking waveform. (b) Slow sucking pattern (Ssk), insert shows a section of the recording played at higher speed revealing rhythmic 0.9 Hz, low amplitude strokes. (c) The mixed slow sucking and salivation pattern, where sucking and salivation signals are much more irregular and of minor amplitude compared to (a). Points in the trace where sucking and salivation were observed are indicated. One min and 10 mV scale bars applicable to traces a, b and c. Separate scales shown for inserts.

of fluid between the capillary tip and the tick's hypostome. Any movements of fluid out of the tick were, however, insignificant in volume compared to the quantity expelled by the tick during pattern 2. This pattern has for the present treatment thus been labelled 'sucking and salivation' (Sk/Sal in Fig. 1c).

Examination of feeding electrograms reveals certain recurring pattern sequences, the most striking of which is the Fsk, Sal and rest patterns which make up the 'feeding-complex' shown in Fig. 1(a). A period of Fsk comes to an abrupt halt with the sudden release of saliva. Vigorous mixing of the capillary contents follows and subsides during the rest phase. A series of very rapid, large-amplitude pulses, usually occurring in groups of three or four, accompany the shift in signal baseline observed at the onset of the next rapid sucking phase. The release of saliva which, on average, accounted for just 40% of the volume taken up during the preceding period of Fsk, could, on occasion, total up to 80% of this volume. For the other patterns, recurring sequences were less clear. Both Ssk and Sk/Sal are of longer duration, but are occasionally interrupted by isolated Sal type releases more normally associated with the feeding complex.

Biopotentials contributing to the feeding complex

As the Fsk, Sal, rest 'feeding complex' is indicative of the highest rates of serum uptake, a more detailed investigation of the electrical events contributing to it was made. The electrophysiological amplifier, as compared to the $10^9 \Omega$ Feeding Electrogram amplifier, permitted in-

vestigation of tick muscle and nerve potentials in the capitulum. Fig. 2 shows all the elements of a 'feeding complex' recorded in this way. Similar recordings were obtained from seven other ticks. Fig. 2(a) is the A.C. or high-pass filtered signal, while the lower trace (Fig. 2b) shows the signal after it has undergone low pass filtering, thereby giving a trace more reminiscent of the conventional feeding electrogram recordings. The Fsk (in this example of 3–4 Hz) phase of the feeding electrogram is characterized by volleys of rapid potentials in the A.C. trace. These volleys, shown in detail in Fig. 2(c), correspond in frequency with the Fsk action of the transilluminated tick viewed under the microscope. The regularly spaced bursts of electrical potentials come to an abrupt stop with the release of saliva indicated in Fig. 2(b) by an arrow. Thereafter, a train of spikes occurs, which is only visible in the A.C. trace. In the ensuing seconds, the spikes increase in size up to a maximum of approximately 6 mV (Fig. 2d). The end of the rest phase and the transition to the next period of Fsk, is marked by cessation of these tonic potentials and by a series of irregular fluctuations in both high and low pass signals, represented in the latter as a series of small drops in signal level. Similar brief drops in the signal level can also occur intermittently towards the end of a period of Fsk. The Fsk pattern is resumed some seconds from the end of this group of 'transition' signals.

Feeding electrogram assay on pluri-infested rabbit serum

Fsk, Sal, rest, Ssk and mixed Sk/Sal patterns are expressed as mean percentages of successive 3 h recording

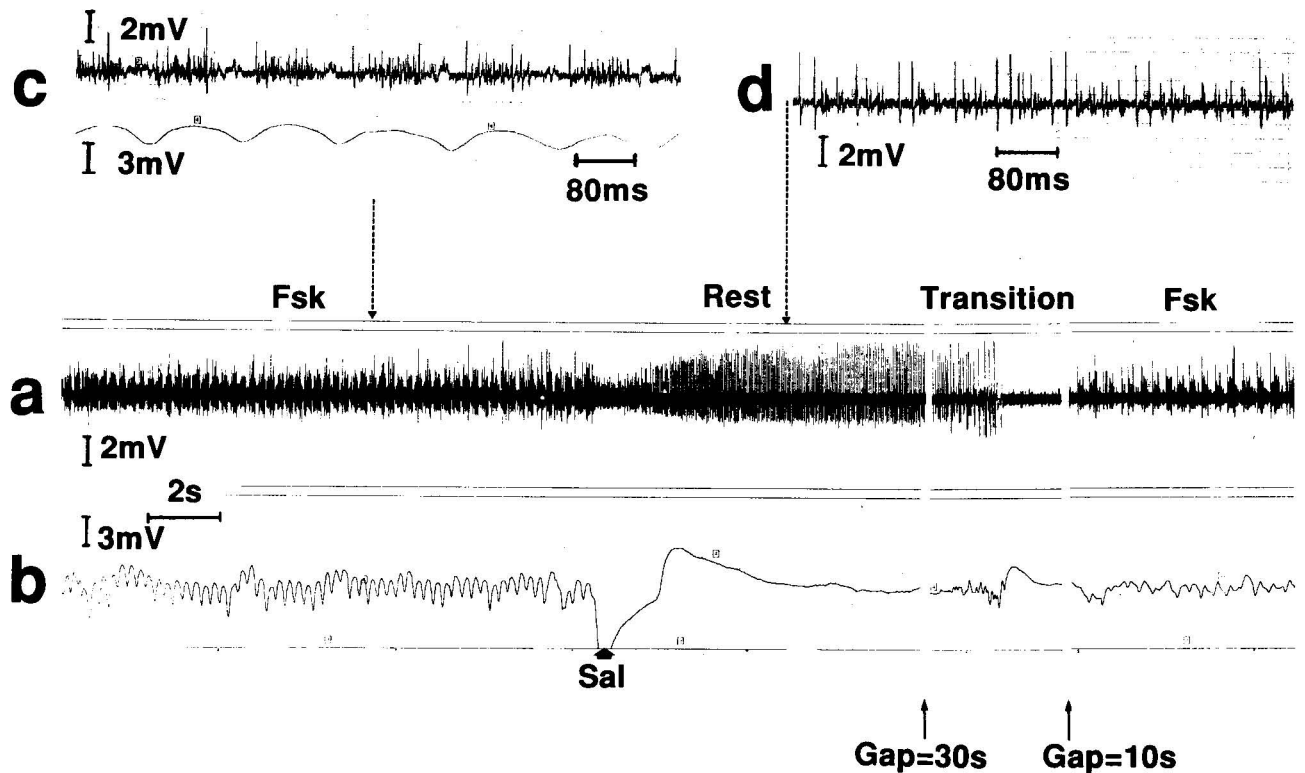


Fig. 2. Electrical potentials generated in the tick capitulum in the course of one ‘feeding complex’ detected with a high input impedance amplifier: (a) high-pass filtered signal; (b) signal after low-pass filtration, but with automatic drift correction of the amplifier in operation (arrow indicates when release of saliva was observed); (c) bursts of high frequency signals which contribute to the 3–4 Hz waveform (detail of (a) and (b)); (d) tonic spike train, maintained throughout the rest period. Cessation of the spike train with the transition to the next bout of fast sucking, is accompanied by a series of more irregular signals with both low and high frequency components. For clarity, the section of trace shown is compressed, with a ‘gap’ of 30s near the end of the rest phase and one of 10s at the start of the second period of fast sucking. For further details see text.

periods, in 9 h ‘bracketing’ experiments, where tick feeding behaviour on naive serum was compared with that on pluri-infested serum (Fig. 3). During the first period of feeding on naive rabbit serum the dominant feature of the feeding electrogram is Fsk accounting for some 50% of the total; Ssk and Sk/Sal patterns together amount to less than 35% of the recording (Fig. 3a). On changing to serum from pluri-infested rabbits, pronounced changes are observed in the total feeding electrogram (Fig. 3b). A significant decrease in the proportion of the trace occupied by Fsk is accompanied by an increase in the percentages of both the Ssk and Sk/Sal patterns. Rest and Sal events showed a significant decrease proportional with the decrease in Fsk.

Replacing the pluri-infested serum with a fresh naive serum-filled capillary effectively reverts the feeding electrogram pattern to that occurring at the outset, where Fsk predominated. No significant difference was found between Fsk, Rest or Ssk events in the first and second periods of feeding on naive serum. However, both proportions of the recording for which Sal and Sk/Sal patterns occurred were significantly lower during the second feed on naive serum. When the same tick-naive serum was offered for all three feeding periods in a separate experiment, no such differences were found.

Mean weight gains of $33.4 \pm 5.0\%$ and $24.8 \pm 4.2\%$, respectively, for first and second periods of feeding on naive sera were significantly higher than the $7.9 \pm 1.5\%$ increase achieved on the pluri-infested sera ($\bar{x} \pm SE$, $n = 30$, $P < 0.001$).

Discussion

The modest weight gains recorded during periods of feeding on capillaries filled with tick-naive sera, suggest that feeding is compromised, even for those ticks chosen here for their ability to feed *in vitro*. The poorer nutritive value of serum compared with whole blood, and the continuous dilution of the limited serum-reservoir by injections of tick saliva are two major factors involved. Despite this limitation, the usefulness of this *in vitro* technique for the screening of test sera, often only available in small quantities, is clear.

The feeding electrogram

Feeding electrogram studies on *Boophilus microplus*

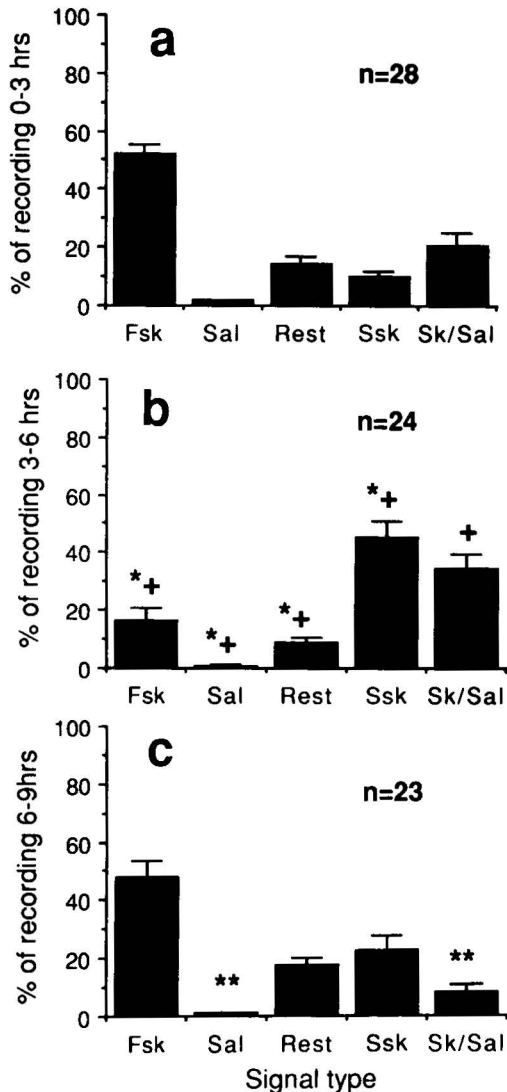


Fig. 3. Successive 3 h feeding electrogram recordings from semi-engorged *R. appendiculatus* females. Distribution (means and standard errors) of fast sucking (Fsk), saliva bursts (Sal), rest, slow sucking (Ssk) and mixed sucking–salivation (Sk/Sal) patterns. Ticks were first fed on ‘naive’ rabbit serum (a), then pluri-infested serum (b) and finally on naive rabbit serum again (c). The histograms are results from separate experiments in which sera from four different rabbits were tested, before (naive) and after two artificial infestations with ticks (pluri-infested). * Pattern frequency in (b) differed significantly from that in (a); + pattern frequency in (b) significantly different from (c); ** pattern frequency in (c) significantly different from (a) ($P < 0.001$ for Fsk, Sal, Ssk and Sk/Sal comparisons, and $P < 0.05$ for rest).

(Gregson, 1967; Tatchell *et al.*, 1972; Waladde *et al.*, 1979), and those by Sweatman *et al.* (1976) on *Hyalomma dromedarii*, Stone *et al.* (1983) on *Ixodes holocyclus*, and Paine *et al.* (1983) on *Dermacentor andersoni* all show strong parallels in basic features of the feeding electrogram with findings presented here on *R. appendiculatus*. In essence, all feeding electrograms represent fluctuating levels of resistance, modulated by the feeding arthropod.

Due to the relatively high impedance of the Feeding Electrogram amplifier used, biopotentials (discussed in more detail below) and physico-chemical effects (e.g. half-cell potentials at the electrode contacts and streaming potentials generated in the mouthparts) also contribute to the recorded signal. Tjallingii (1985) points out that the performance of amplifiers and chart recorders used to detect the signal, can have a major effect on the nature of the recording. This may account for many of the differences in the form of recordings in the studies cited above. Feeding electrograms for the tick made *in vivo* and *in vitro* show good agreement (Stone *et al.*, 1983). Likewise, there was good agreement between feeding patterns described here for *R. appendiculatus* feeding from capillaries and those simultaneously recorded from ticks feeding through an artificial membrane on a static pool of serum (Lösel, unpublished). There is nothing to suggest therefore that the behaviour observed is simply an artefact of the feeding method.

The ‘feeding complex’ first described for *H. aegyptium* by Sweatman & Gregson (1970), for *Boophilus microplus* by Tatchell *et al.* (1972) and Waladde *et al.* (1979), and that described here for *R. appendiculatus*, assumes the form of a very low frequency square-wave. The two distinct levels of the signal associated with sucking and with salivation leading on to rest periods, demonstrate sudden changes in electrical properties of the preparation, above all a change in the resistance. This change, shown as a shift in the potential recorded between periods of sucking and salivation, is due to an altered electrical pathway between the reference electrode, in contact with the haemolymph, and the recording electrode connection to the tick’s interior through the serum reservoir. During sucking, it is the gut which effectively forms the barrier between the electrodes, while during salivation, it is the walls of the salivary acini. Tatchell *et al.* (1972) suggested that during feeding the gut offers a relatively higher resistance than does the salivary system during salivation. Differences in the structure of these organs, in particular the enormous surface area of the salivary glands of partially fed ticks, and also in the relative conductivity of the fluids they contain, will influence their electrical resistance.

The causes of these changes in electrical properties of the preparation lie in the anatomy and disposition of the feeding apparatus. Morphological studies of the hard-tick’s feeding and salivary apparatus (Gregson & Tatchell, 1971; El Shoura, 1988) suggest that the key elements of the ‘switch’ between resistances are opening and closing of the pre-pharyngeal valve, and lowering and raising of the salivarium floor. Functionally, the salivarium floor acts as a salivary release valve (El Shoura, 1988). During feeding it is in a raised position, stopping the escape and ingestion of saliva, while the pre-pharyngeal valve is open. During salivation the latter is closed ensuring that the secreted saliva leaves the tick, guided by the salivarium floor, and is not passed into the gut.

The signal categories forming the complex of fast sucking, burst-like saliva release and rest, are the key elements of the tick’s response to different food substrates. Although

weight gains were recorded during periods of slow sucking, and mixed sucking–salivation patterns, the fast sucking pattern had a determining role on the weight gains achieved. While saliva excretion results in a reduction in tick weight, the number of burst-like salivations is an indication of how able the tick is to assimilate food taken in.

Although the precise location of the electrode-tip is unknown, recording from the tick capitulum with an electrophysiological amplifier, shed some further light on the control of the feeding electrogram resistance changes contributing to the feeding complex discussed above. During fast sucking, regular volleys of rapid electrical potentials, which corresponded in frequency to the sucking action observed under the microscope, are probably action potentials associated with the rhythmic contraction of the pharyngeal dilator muscles. These muscles distend the pharyngeal lumen to create a negative pressure which draws serum in. As the electrical resistivity is inversely related to cross-sectional area of a current-carrying conductor, in this case the serum-filled pharyngeal lumen, resistance modulations of the fast sucking waveform are a direct result of changes in the cross-sectional area of the pharyngeal lumen. Separating the volleys of regular electrical activity associated with serum uptake, are short electrically quiet phases, probably corresponding to passive periods in which the pharyngeal dilator muscles relax. The pharynx then decreases in volume and, aided by closure of the pre-pharyngeal valve and contraction of circular muscles enveloping the pharynx (Balashov, 1972), serum or blood is propelled into the oesophagus.

The very uniform spikes which occur uniquely and tonically throughout the rest phase of the feeding electrogram, are almost certainly nerve cell potentials. Their absence during the sucking phase shows that they are in some way linked to the release of saliva. Kemp (1978) presents a trace similar to the high pass filtered signal of the present study, for recordings made from the pre-pharyngeal valve muscles of *Boophilus microplus* feeding *in vitro*. Kemp describes a one-to-one relationship between these 'spikes' and the twitching of the pre-pharyngeal valve, the hypothesized function of this action being to expel saliva. Tatchell *et al.* (1972) refer to the whole period between consecutive feeding bouts as 'salivation', implying that saliva flows out of *Boophilus* throughout this period. In the present study with *R. appendiculatus* neither twitching of the valve nor escaping saliva was observed. In fact, no fluid movements at all could be discerned in the capillary tube during rest, after the burst of saliva had passed. The relatively high frequency of the signals (around 30 spikes/second in this case), makes serial opening and closing of the valve seem unlikely. An alternative explanation for these spike trains observed during the release of saliva and the ensuing rest phase, is that they originate from the motor innervation of muscles controlling the lowering of the salivarium floor, in its capacity as a saliva-release valve mentioned above. As opening of the salivarium would require sustained muscle contraction, involvement of the tonic spike train found

here appears quite plausible. If on the contrary these action potentials were associated with closure of the pharynx, one would expect to see the spikes, and the large changes in resistance which immediately precede and follow the spike-train, coincide with the sucking action. The direct observations showed that this clearly was not the case.

The irregular signals seen at the transition from the rest phase to the fast sucking pattern and occasionally also towards the end of a fast sucking bout, may represent repeated, but incomplete opening and closing of the salivary valve as discussed above. Similarly for *D. andersoni* Gregson (1967) describes 'fluttering' of this structure. Certainly, the oscillation of the trace between the two base-lines would be consistent with the interpretation of the latter structure having a role analogous to an electrical 'switch' in the tick feeding electrogram preparation.

Effect of pluri-infested sera on feeding behaviour

Reviewing the immunological basis of host resistance, Wikel & Allen (1982) distinguish between that which is innate to the host and that which is induced by contact with an ectoparasite. Here, resistance is of the latter type. Tick resistance is frequently gauged by the decrease in female engorgement weights. Mean weights of *R. appendiculatus* in the present study decreased from 401.3 mg through 195.6 mg to 61.6 mg for first, second and third infestations respectively, demonstrating considerable tick resistance already after only two infestations. Passive transfer of resistance by inoculating naive hosts with sera from pluri-infested ones has been established for guinea-pigs infested with *Dermacentor variabilis* larvae (Trager, 1939) more recently, for rabbits infested with *Ixodes ricinus* (Brossard & Girardin, 1986) and for *R. appendiculatus* (Rubaire-Akiki & Mutinga, 1980). These studies demonstrate that effectors of the tick-resistance are, at least in part, humoral (Wikel & Whelen, 1986). Kemp *et al.* (1986) found that resistance induced in cattle by injection of 'concealed' *B. microplus* mid-gut antigens was expressed against ticks feeding *in vitro* on serum from the same animals. These authors suggested that complement may be involved in the resistance mechanism since heat-treatment of the sera could reduce the strength of the effect. Heat-treatment of the pluri-infested serum used in this study also tended to increase the weight gains of both partially engorged and unfed ticks, raising the possibility of similar effectors being involved here. Damage to the gut basement membrane observed by Walker & Fletcher (1986) in *R. appendiculatus* which had fed on resistant hosts suggests that other mechanisms may also be involved *in vitro*.

The bracketing experiments, where individuals were fed for relatively short periods on both tick-naive and pluri-infested sera, demonstrate that the tick responds quickly to the quality of the serum it is offered. The literature concerning tick *in vitro* feeding experiments provides various precedents. Waladde *et al.* (1979) demonstrated in feeding electrogram studies that addition of the tick

phagostimulants ATP or GSH to saline led to rapid increases in sucking behaviour. Conversely, Paine *et al.* (1983) showed that within minutes of adding histamine to the diet of *D. andersoni* adults, there was a decline in the amplitude of the sucking waveform recorded. Waladde & Rice (1982) state that ticks fed on resistant blood through an artificial membrane altered feeding behaviour and caused rapid detachment.

Although effects of these, as yet unidentified, serum-bound resistance factors on the metabolism of the tick cannot be ruled out, the studies cited above, and the relatively fast response of the tick to the different serum types in the bracketing experiments presented here, all suggest a behavioural response. This is possibly mediated by the peripheral sensory system of the tick. Studies by Waladde & Rice (1977) for *B. microplus* and Sonenshine *et al.* (1984) on *D. variabilis* show that gustatory pit sensilla and mechano-receptors are present on inner and outer cheliceral denticles. Scanning and transmission electron microscope studies on the chelicerae of *R. appendiculatus* in this laboratory confirm the presence of chemoreceptor pit sensilla showing abundant innervation. Stimulation of the same sensilla with various salt solutions and sera, elicits complex multi-cell responses. Cone depressions with innervation typical of mechanoreceptors are also present. The tick is therefore equipped to sense chemical and physical characteristics of the environment at the anterior extremities of its mouthparts, although as Waladde & Rice (1982) point out, the possibility of gustatory sensilla being sited in more posterior regions of the food tract cannot be excluded.

Increases in acinus diameter and number of secretory granules in 'C₁' cells of salivary gland type II acini, were observed for partially engorged female *R. appendiculatus* fed on rabbits, an unusual host, above those observed for ticks feeding on the usual bovid host (Walker & Fletcher, 1990). Further increases of both granular contents and volume of acini were recorded by these authors for ticks fed on rabbits which had previously been infested. The lack of change found by these workers in acinar volume and the much smaller increase in secretory granules for ticks fed on pluri-infested bovinds, further demonstrates the tick's capacity to react to the quality of its food substrate. It also correlates well with low tick-resistance of pluri-infested cattle compared to the high resistance of pluri-infested laboratory rabbits (Walker & Fletcher, 1986). The secretion of potent immunoregulatory substances in tick saliva (Ribeiro & Spielman, 1986; Ribiero *et al.*, 1990) suggests that the tick may not only be able to sense, but may indeed be able to manipulate immune responses of its host. These differences in the immune responses of rabbits and bovinds to *R. appendiculatus*, may explain the relative lack of a difference observed in the *in vitro* feeding response of this tick species when fed on naive and on pluri-infested bovine sera in the current study, compared with the marked difference to naive and pluri-infested rabbit sera. Since, in the long run, the strong immune response of the rabbit to this unusual parasite may be

maladaptive, possibly leading to self-inflicted injury, the much milder immune response of cattle may be sufficient to affect the tick population as a whole over time without incurring such acute self-inflicted injury.

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