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Cuticle expansion during feeding in the tick *Amblyomma hebraeum* (Acari: Ixodidae): The role of hydrostatic pressure

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ABSTRACT

Female *Amblyomma hebraeum* ticks (Acari: Ixodidae) increase their weight ~10-fold during a 'slow phase of engorgement' (7–9 days), and a further 10-fold during the 'rapid phase' (12–24 h). During the rapid phase, the cuticle thins by half, with a plastic (permanent) deformation of greater than 40% in two orthogonal directions. A stress of 2.5 MPa or higher is required to achieve this degree of deformation (Flynn and Kaufman, 2015). Using a dimensional analysis of the tick body and applying the Laplace equation, we calculated that the tick must achieve high internal hydrostatic pressures in order to engorge fully: greater than 55 kPa at a fed:unfed mass ratio of ~20:1, when cuticle thinning commences (Flynn and Kaufman, 2011). In this study we used a telemetric pressure transducer system to measure the internal hydrostatic pressure of ticks during feeding. Sustained periods of irregular high frequency (>20 Hz) pulsatile bursts of high pressure (>55 kPa) were observed in two ticks: they had been cannulated just prior to the rapid phase of engorgement, and given access to a host rabbit for completion of the feeding cycle. The pattern of periods of high pressure generation varied over the feeding cycle and between the two specimens. We believe that these pressures exceed those reported so far for any other animal.

1. Introduction

The blood meal of female ticks (Acari; Ixodidae) is conventionally divided into three phases. In a preparatory phase, the tick cements itself to the skin and prepares the feeding lesion. The slow phase of engorgement lasts about 6–7 days, during which the fed: unfed mass ratio (hereafter called "mass ratio") reaches about 10; this is followed by the rapid phase (about 12–24 h) during which the mass ratio can approach 100 (Kaufman, 2007).

The mass of cuticle available to the unfed female is insufficient to contain the body volume at the end of engorgement. In order to meet this demand, the endocuticle increases about 40% in thickness during the slow feeding phase. Although the tick grows some additional endocuticle during the rapid feeding phase, because of rapid expansion at this time, the cuticle thins by about 50% (Flynn and Kaufman, 2011). Given that the width-to-length ratio of the tick remains constant (0.77) throughout the feeding cycle, this requires that the cuticle plastically (permanently) deform by more than 40% in each of two orthogonal directions (Flynn and Kaufman, 2011, 2015.) An analysis of deformation vs. stress indicates that a stress level of 2.5 MPa must be reached in the cuticle before deformation of 40% or more can be achieved, and suggests that the neurotransmitter dopamine (DA) plays an enabling role, likely through a modification of cuticular pH (Flynn and Kaufman, 2015).

The Laplace equation relates internal pressure in a vessel to the stress in the wall. For a cylindrical shape, the pressure required to induce a given circumferential (hoop) stress is given by:

$$P = \sigma * t/r$$

where P is pressure (Pa), σ is circumferential wall stress (Pa), t is thickness (m), and r is the radius (m) (Vogel, 2003). For a spherical vessel, the pressure required to achieve a given wall stress level is twice that for circumferential stress in a cylinder.

We have previously modeled the tick as an ellipsoid (Flynn and Kaufman, 2011); the pressure/stretch relationship for such a shape would lie between that of a cylinder and a sphere. Fig. 1 shows the required pressure to achieve a wall stress of 2.5 MPa for a cylinder and sphere, based on dimensions for a feeding female *A. hebraeum* in the mass ratio of 20–100 (Flynn and Kaufman, 2011). Predicted pressures, in the range of 55–110 kPa at a mass ratio of 20, are well above those observed in mammalian circulatory systems, and in a previous study of the internal hydrostatic pressure of an argasid

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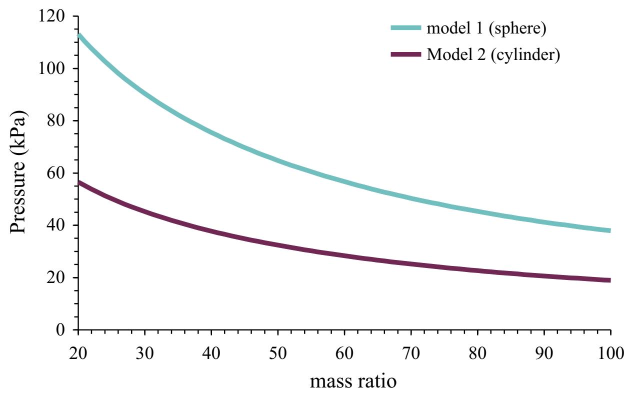

Fig. 1. Pressure (*Y*-axis) required to achieve a wall stress of 2.5 MPa in a cylinder and a sphere for ticks the size of *A. hebraeum*, as a function of mass ratio. A plot for the approximate ellipsoid shape of the tick would fall somewhere between these two curves.

tick during feeding (Kaufman et al., 1982). To confirm whether such high pressures could be generated within the feeding ixodid tick, we cannulated ticks and connected them to a telemetric pressure transducer, as described in Section 2.

2. Materials and methods

The tick colony was maintained in darkness at 26 °C at a RH exceeding 95%. For feeding ticks, a cloth-covered foam arena (~12 cm × 8 cm × 2.5 cm) was glued to the shaven back of a rabbit with a latex adhesive. Prior to cannulation, ticks were partially-fed to about 10× the unfed mass (about 6 days) on laboratory rabbits, as described by Kaufman and Phillips (1973). The use of rabbits was approved by the Biosciences Animal Policy and Welfare Committee, University of Alberta, which functions under guidelines established by the Canadian Council on Animal Care.

2.1. Cannulation procedure (Fig. 2)

For measuring internal hydrostatic pressure, partially fed ticks were removed from the host and cannulated with either microrenathane MRE-40 tubing (Braintree Scientific Inc., Braintree MA, USA) or polyvinyl V3/A tubing (Scientific Commodities Inc., Lake Havasu City, AZ, USA); the cannula had been pre-filled with a solution of 8 mg polyvinylpyrrolidone/10 ml heparinized saline. To insert the cannula, a puncture wound through the cuticle was made with a 20 g hypodermic needle at the lateral margin of the opisthosoma in the vicinity of the spiracle. The tip of the cannula was inserted through the puncture wound (the fit was snug) approximately 5 mm into the haemocoel, and was sealed in place with Silastic Medical Adhesive Silicone (Dow Corning, Midland, Michigan, USA). The end of the cannula distal to the tick was connected to a PA C-10 or C-40 telemetric pressure transducer (Data Sciences International, St. Paul, MN, USA). After allowing the adhesive to set for a few hours, the cannulated ticks were returned to the host rabbit, and the transducer was lightly taped to the rabbit's skin to inhibit it from rolling around as the rabbit moved. For pressure measurements, the rabbit was placed in a small pen (about 100 cm × 70 cm) made of radio-transparent walls. Pressure signals from the transducer were monitored using the PhysioTel system (Receiver model RPC-1) and recorded on a laptop computer using LabChart version 7.2.5 software (AD Instruments, Colorado Springs, CO, USA). Because of the limited transmission range of the transducer, two or three receivers were mounted on the walls of the pen so as to optimize signal reception. The maximum amplitude that could be recorded by the telemetric pressure transducer was ~57 kPa. MathWorks Matlab Fast Fourier Transform software was used to analyze the pressure trace of one of the ticks (Ah 266).

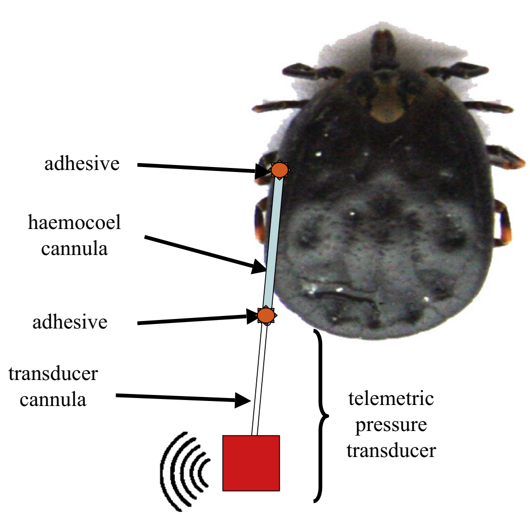

Fig. 2. The procedure for cannulating the *A. hebraeum* ticks to the telemetric pressure transducer. The tick shown is at the approximate mass ratio (~10) at which all the ticks were cannulated. The tip of the haemocoel cannula lodges approximately 5 mm beyond the point of penetrating the cuticle. The adhesive forms a seal to prevent leakage of haemolymph and to withstand the pressure generated. See Section 2 for further details.

3. Results

For this study we cannulated 16 female *Amblyomma hebraeum* ticks. Two of the 16 never re-attached to the host. Three died on the host before meaningful data could be recorded. One was deemed invalid because of technical problems with the recording system that day. Three were ticks that had been killed by freezing and then thawed before cannulation; these ticks were to serve as a control to distinguish pressure generated by the tick as opposed to pressure generated on the transducer by movements of the host rabbit. In three cases the cannula disengaged from the tick at some point during the run. Four ticks did feed to engorgement. However, in two of these four (Ah 208 and 211), the cannula was probably blocked, perhaps because the cannula damaged the delicate gut wall causing a release of gut contents; the recording showed very little spontaneous activity, and when the tick was squeezed at the end of the run there was very little or no response by the transducer. Hence valid pressure recordings during rapid engorgement are available from two ticks: Ah 254 and 266. Note that the time of reattachment of the tick to the rabbit was uncertain for both ticks, so the exact relation of the time of pressure recording to the feeding cycle cannot be determined. The pressure traces can be thought of as a window into an interval of a longer process.

The control ticks showed infrequent small pressure "blips" that are likely related to the movement of the rabbit; one instance of 25 kPa was observed, all others were less than 12 kPa, and no repeated period of pressure generation was observed; i.e. the pressure blips were isolated events.

Pressure readings for Ah 266 were recorded at 1000 Hz, for 21.5 h. Repeated periods of high frequency high pressure (HFHP) pulses were observed; Fig. 3 shows three traces of pressure vs. time over a one second period. Note that 57.5 kPa was the maximum observable pressure from the experimental setup; it is likely from various instances of flat-lining of the peaks that internal pressure in the tick exceeded this value. The negative pressure values may reflect recoil of fluid in the tubing between the tick and the pressure transducer. Here we define a 'high pressure spike' as any pulse