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PROTECTION OF ANIMALS FROM TICKS BY INTERFERENCE IN THE FEEDING PROCESS OF TICKS

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This project was designed to test the possibility of blocking tick's cheliceral chemoreceptor with specific antibodies. This approach was based on the assumption that a specific chemostimulation of this receptor is essential for initiation of the blood gorging response. As previous experiments had demonstrated, this assumption holds true for the soft tick Ornithodoros tholozani; however, its close relative, O. moubata, feeds to some extent in the absence of such stimuli. Thus, before going ahead with the planned anti-receptor immunization, we conducted a detailed comparison of the feeding response between these two species.

In order to obtain the optimal feeding conditions we used a special membrane (developed by our Kenyan collaborator; see previous report) which was made similar to the natural rabbit skin by glueing rabbit hair and applying rabbit ear wax to the membrane upper surface. The addition of ear wax made attachment more rapid, thus leading to a higher % of ticks reaching engorgement during the 90 min. experimental time. For O. tholozani , the addition of ear wax increased the % response to Glutathione (GSH) 10^{-3} M from 10 to 80%. We used nymphal ticks which were starved for 5-7 months in order to ensure good appetite. Fresh rabbit blood was used as a positive control. To allow careful monitoring of tick's feeding behavior and avoid possible crowding influences, ticks were fed individually. Nymphs used had unfed weight of 5 mg; ticks with repletion weight of 10

mg and above, were considered as fed. Glutathione and Adenosine Triphosphate (ATP) both had been reported to stimulate feeding in ticks, were used in this study. These compounds were diluted in saline containing glucose (1 mg/ml) and sodium bicarbonate 10^{-3} M (SGB). The SGB diluent was used to simulate blood conditions and was found, in previous experiments, to enhance the feeding response of ticks to GSH and ATP.

Table 1 gives the results obtained in this study. While O. moubata fed on saline and SGB, O. tholozani, did not. For O. moubata, GSH at 10^{-3} M was the only substance that induced a feeding response significantly higher than that of the SGB alone. ATP at 10^{-3} M seem to exert an inhibitory effect on O. moubata. O. tholozani fed best on GSH at 10^{-3} M and ATP at 10^{-3} M. Repletion weights had correlated directly with the % feeding response. Feeding on blood leads to the highest weight gain in both species.

For O. tholozani a solution containing glucose like SGB did not stimulate any feeding while 60% of O. moubata fed on SGB. This indicated to us that glucose (at 1 mg/ml) acts as an independent phagostimulating compound for O. moubata. Therefore we tested again the response of O. moubata to GSH and ATP in the absence of glucose. Results are given in Table 2. At the optimal stimulating concentration of GSH and ATP (10^{-3} M and 10^{-5} M, respectively) the absence of glucose did not affect the feeding response. However, when less than optimal concentrations were used, glucose does seem to enhance feeding.

Thus, O. moubata feeds quite effectively on diets which do not contain the major stimulus GSH, even though it possesses receptors which recognize GSH. It is therefore very likely that blocking the GSH receptors in this species would not interfere with their engorgement process.

We have to find out what the dependence is of Ixodid ticks on phagostimulants before we concentrate on isolation of their receptor proteins. For that pupose we tested the suitability above described membrane for Rhipicephalus sanguineus and found that prefed adult females attach to the membrane and engorge blood. This system will be used to test the role of phagostimulants for hard ticks.

Table 1. Comparison of feeding response between *O. tholozani* a
*O. moubata** to various concentrations of GSH and ATP

Material tested	*O. tholozani* n	% fed	weight gain mg + 50	*O. moubata* n	% fed	weight gain mg + 50
Blood	28	100	31 ± 17	20	85	21 ± 3
Saline	20	0	-	57	30	15 ± 4
SGB+	20	0	-	64	66	16 ± 6
GSH 10^{-3} M	105	77	25 ± 10	28	86	14 ± 5
GSH 10^{-4} M	15	33	26 ± 8	26	69	17 ± 6
ATP 10^{-3} M	41	44	22 ± 10	18	44	11 ± 4
ATP 10^{-4} M	34	24	17 ± 8	27	63	13 ± 4
ATP 10^{-5} M	34	21	18 ± 15	23	70	15 ± 5

* Nymphs, 5 mg unfed weight, fed individually on an artifici
membrane

\+ Saline with glucose (1 mg/ml) and sodium bicarbonate 10^{-3} I
used as diluent for GSH and ATP

Table 2. Feeding response of *O. moubata** to GSH and ATP in the absence and presence of glucose+

	without glucose		with glucose	
	n	% fed	n	% fed
SB	53	13	64	66
GSH 10^{-3} M	34	95	28	86
GSH 10^{-4} M	33	40	43	67
ATP 10^{-5} M	33	67	23	70

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* Nymphs, 5 mg unfed weight, fed individually on an artificial membrane.

\+ 1 mg/ml.

PROGRESS REPORT FROM ICIPE

INTRODUCTION

Tick gustatory receptor proteins are located in the cheliceral digits. They are chemoreceptors stimulated by phagostimulants components in the host blood. Two phagostimulants are involved in the stimulation of cheliceral receptors. These are glutatione (GSH) and Adenosine tri phosphate (ATP) in combination with glucose.

Progress of Work

Chedmiceral digits from both male and female Rhipicephaslus appendiculatus have been harvested in cold (4°C) phosphate buffered saline pH 7.2 containing 1 mM EDTA and protease inhibitors (1 mM PMSF). Different extraction methods have been performed in order to asess the amount of protein extracted. Sonication and homoginization methods released the highest amount of protein. Addition of detergents trixon x 100 and deoxycholic acid added at 1% final concentration increased further the amount of the protein extracted. The purpose of this exercise was to maximize the amount of protein extracted from the cheliceral digits (Table 1).

Figure 1 shows the analysis of proteins extracted in sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE). Using a 10% SDS-PAGE stained with coomassie blue, between 1 to 10 polypeptides with relative molecular mass of 94 to 14 KD were

resolved. Both male and female ticks gave similar protein profile. Example of the profile using female ticks is shown (Figure 1). Efforts are being made to identify which of this polypeptide bands bind to labelled GSH or ATP.

Future

Identification of the polypeptide bands binding to labelled GSH or ATP will intensify. Possibly the identity of chelicéral receptor protein will be achieved. Further work on identity of other or related proteins is also in progress. It is anticipated that gustatory receptor proteins or similar proteins may be identified in the gut of the tick. Previous work with mid-gut of Rhipicephalus appendiculatus have shown that protein extracts of this organ confer immunity to rabbit, goats and sheep (ICIPE Annual Report 1985, 1986 & 1987). It is therefore not remote to anticipate the presence of gustatory receptors in these tissues. It is possible to postulate the isolation of gustatory receptor proteins using Affinity Chromatography. The ligand coupled will be GSH or ATP alternatively. Monoclonal antibodies (MAB) may be produced to the proteins eluted from GSH or ATP affinity column. These MAB can then be used in further purification and immunochemical characterization of gustatory receptor proteins.

Figure 1. SDS-PAGE Analysis of Rhipicephalus appendiculatus female tick cheliceral digits proteins. LANE A: Molecular weight protein marker* LANE B: Extraction by homoginization and sonication LANE C: homoginization only LANE D: Sonication only LANE E: Homoginization in presence of detergent. LANE F: Homoginization and sonication in presence of detergens.

^Pharmacia low molecular weight markers.,

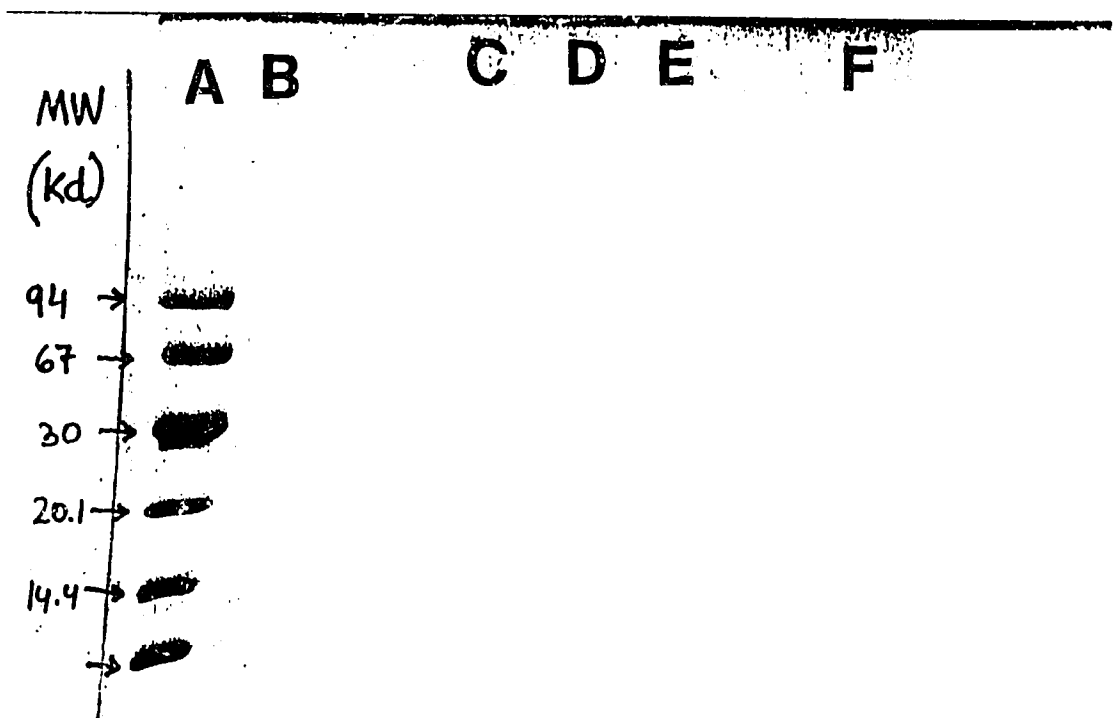

Table 1. Amount of protein extracted from the cheliceral digits of *Rhipicephalus appendiculatus* using different extraction methods

Source	Extraction method				
	H	S	Hs	HD	HSP
Female	130	90	250	260	300
Male	110	70	200	250	250

Key: Protein in ug/ml (200 cheliceral digits in 1 ml) content estimated by BIO-RAD protein assay procedure.

H = Homoginization

S = Sonification at 300 watts pulses for 1 min

HS = Homoginization and sonication

HD = Homoginization in presence of detergents

HSD = Homoginization and Sonication in presence of detergents.