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RESPONSES OF CATTLE TO ALLERGENS FROM *BOOPHILUS MICROPLUS*

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Abstract—WILLADSEN P., WILLIAMS P. G., ROBERTS J. A. and KERR J. D. 1978. Responses of cattle to allergens from *Boophilus microplus*. *International Journal for Parasitology* **8**: 89–95. Extracts of larvae of the cattle tick, *Boophilus microplus*, contain two allergens giving immediate hypersensitivity reactions in the skin of cattle which have been exposed to the tick. The partial purification of one of these is described. This, together with the other allergen whose purification has been described previously, was used to investigate the relationships between tick resistance, immediate hypersensitivity reactions, reaginic antibody levels as measured by Prausnitz–Küstner type reactions and serum levels of agglutinating antibody to one of the allergens. There were significant correlations between resistance and both the immediate hypersensitivity reactions and agglutinating antibody levels.

INDEX KEY WORDS: allergen; antibody; *Boophilus microplus*; immediate hypersensitivity; Prausnitz-Küstner; tick.

INTRODUCTION

ALL CATTLE on first exposure to the tick *Boophilus microplus* have an initial period of susceptibility during which a high proportion (about 50%) of larvae which attach to the host will successfully complete their life cycle. This period is followed rapidly by the development of resistance. After a host has received considerable exposure to ticks, the proportion of larvae which will complete their life cycle is both characteristic of a particular host and also relatively constant over a long time period (Roberts, 1968*a*). For a highly resistant animal, the proportion will be less than 1% and for a low resistant one, 20% or more. The fact that resistance is acquired is evidence that it is immunologically-mediated, but the immunological reactions responsible remain unknown. In fact, few immunological reactions have been observed in this host–parasite system and, where they do occur, their relevance to resistance to the parasite is not clear. A major loss of tick larvae from British breed cattle occurs within the first 24 h after attachment of the tick (Roberts, 1968*b*) and, within this time, larvae attach and detach repeatedly (Kemp, Koudstaal, Roberts & Kerr, 1976).

Two reactions which have been observed in the *Bos taurus* – *B. microplus* system and which could contribute to such behaviour are a humoral antibody response and an immediate hypersensitivity reaction to tick antigens. Thus Brossard (1976), using immunoelectrophoresis and indirect immunofluorescence, showed that bovine sera contained two precipitating systems against *B. microplus* components. One of these was non-specific, since it also occurred in cattle which had no exposure to ticks. Although it was suggested that the precipitating antibodies were responsible for resistance, there was no demonstration of a correlation between degree of resistance and antibody titre to justify this suggestion. The involvement of a serum component in resistance is indicated however by the results of Roberts & Kerr (1976) who showed that the transfer of plasma from highly resistant cattle to unexposed recipients conferred a small but significant degree of resistance. The second immunological reaction which has been observed is an immediate hypersensitivity reaction at the site of tick attachment which, in extreme cases, causes considerable serous exudation during tick infestation (Riek 1956, 1962). An oedematous dermal reaction is also observed on injection of tick extracts into a host (Riek, 1962). Again, however, the significance of hypersensitivity in tick resistance is unknown. Although it has been suggested that hypersensitivity is both the cause of tick resistance (Riek, 1956, 1962) and a factor favourable to the parasite (Tatchell, 1969) little evidence has been presented for either view.

Any investigation of the immunological basis of resistance to ticks will be complicated if the only test material available is a crude tick extract containing a mixture of antigens. In the work to be described, we have investigated primarily the association of immediate hypersensitivity with

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tick resistance. There are at least two components in an extract of tick larvae which produce immediate dermal reactions in cattle which have been exposed to the tick. The purification of one of these components, allergen 1, and its characterisation as a serine esterase have already been reported (Willadsen & Williams, 1976; Willadsen, 1976).

In this work, we have firstly partially purified the second component, allergen 2. The term 'allergen' is used in this context to denote an antigen capable of eliciting an immediate skin reaction (Stanworth, 1973; Berrens, 1971) and not necessarily one reacting with a bovine IgE. These two allergens have then been tested on several herds of *Bos taurus* and *Bos taurus* × *Bos indicus* with a wide range of resistance levels for variations in the immediate hypersensitivity response and for several serum reactions, to determine the relationship between these reactions and resistance.

MATERIALS AND METHODS

Materials. The source of tick larvae and the purification of allergen 1 have been described previously (Willadsen & Williams, 1976).

Assessment of resistance levels of cattle. The cattle in herd 1 were artificially infested twice with 20,000 *B. microplus* larvae, held in tick free pastures and the female ticks engorging between 20 and 23 days after infestation were counted. The results were calculated as a percentage of the possible tick yield. The procedure has been described elsewhere (Wharton & Utech, 1970; Wharton, Utech & Sutherst, 1973). Herds 2 and 3 were kept in normal field conditions and the ticks from natural infestations were counted periodically. For herd 2, the total tick numbers for 12 successive counts immediately preceding the experiment were used as an indication of tick resistance. For herd 3, the tick numbers were from three counts.

Immediate hypersensitivity tests. These were performed as described previously (Willadsen & Williams, 1976). Briefly, 0·1 ml dilutions of the material to be tested were injected intradermally into clipped skin along the side of the animal and, after 20 min, the size of the oedematous reaction was measured in two directions at right angles and the product was taken as representing the area. These areas were proportional to the logarithm of the amount of material injected, as is shown in Fig. 2. During allergen purification, activities of different fractions were expressed relative to the crude extract for the calculation of allergen yields. Immediate hypersensitivity reactions were assessed with eleven concentrations of each allergen in each animal, the maximum amount injected being 1 μg and the minimum amount 10 pg. For each animal and each allergen, the regression of area of oedematous reaction and the log of the allergen dose was used to calculate the allergen doses necessary to give specified reaction sizes.

Prausnitz–Küstner reactions. Calves from an area free of *B. microplus* were purchased at the age of 1–2 weeks. They were raised free of ticks and helminth parasites and used when about 3 months old. Serum samples of 0·5 ml from both the test animals and adult animals with no tick experience were injected intradermally using both undiluted serum and 1 in 5, 1 in 25 and 1 in 125 fold dilutions of serum in phosphate-buffered saline. Four days later, following intravenous injection of 4 ml of 5% Evans Blue per 10 kg body weight, the injection sites were injected with 0·1 ml of allergen containing 2 μg of protein. The calves were killed after 1 h and the diameters of the reactions were measured on the dermal side of the skin. Between 20 and 25 sera were tested in each calf.

Indirect haemagglutination tests. Antibody titres in the exposed and unexposed animals were determined using the tanned cell indirect haemagglutination technique (Herbert, 1973). Fresh sheep red blood cells were tanned with 1/10000 (w/v) tannic acid and 1 ml of a 5% suspension of tanned cells was coated at pH 6·4 by incubation with 210 μg of the purified allergen 1 in 5 ml PBS at room temperature for 2 h (210 μg of purified antigen had $5{\cdot}3 \times 10^{-2}$ units of esterase activity). Cells were washed with 0·5% BSA in PBS and titrated against serial 2-fold dilutions of sera which had been heated for 30 min at 56°, then absorbed with tanned red blood cells. The highest dilution which agglutinated was taken as the endpoint. Control incubations were carried out using uncoated tanned cells. Endpoint titres are given as $\log_2$ of the endpoint dilution. Half values occur in the haemagglutination data in the text since they are the average of two determinations. Duplicates normally agreed to within one dilution.

Determination of protein concentration. Protein concentrations were measured by the UV absorbance at 280 and 260 nm (Layne, 1957).

RESULTS

Purification of allergen 2

A typical purification will be described. One hundred grams of tick larvae were homogenized in a mortar, after pre-cooling both mortar and larvae to −20°C. They were then extracted in 0·05 M-phosphate buffer, pH 7·5, using 2·5 ml buffer per gram of larvae. After centrifugation, the supernatant was fractionated between 50 and 65% ammonium sulphate saturation and the precipitate was dialyzed against 0·05 M-Tris buffer, pH 8·7. This solution was loaded on to a 2·5 × 30 cm column of DEAE cellulose and washed through with 0·05 M-Tris buffer pH 8·7, 0·05 M in sodium chloride. It is necessary to use 0·12 M-sodium chloride to elute allergen 1 from DEAE cellulose at the same pH (Willadsen & Williams, 1976). Four hundred and sixty ml of eluate was collected and concentrated on an Amicon PM-10 membrane, dialyzed against 0·05 M-acetate buffer, pH 4·5 and loaded on a 2·5 × 30 cm column of CM cellulose. The column was washed in the same buffer and then a 0–1 M-sodium chloride gradient was applied. The allergen eluted at a salt concentration of 0·2 M. The most active fractions were pooled and used as the test allergen in the present study. The preparation is summarized in Table 1. The yields and purification factors have been based on the allergenic activity in the original extract. As the original extract contains more than one allergen, the yields and purification factors calculated on this basis are artificially low.

Further purification is possible. Figure 1 shows the chromatography of 0·2 mg of the allergen on a

TABLE 1—PURIFICATION OF ALLERGEN 2

Fraction	Total protein (mg)	Yield of allergen (%)	Purification factor of allergen
Crude extract	9730	100	1·0
0–50% $(NH_4)_2SO_4$	7350	22	0·3
50–65% $(NH_4)_2SO_4$ after dialysis	655	27	4·0
DEAE eluate, after concentration	75	6·0	7·8
Allergen 2, after CM cellulose chromatography and concentration	4·9	1·4	28·0

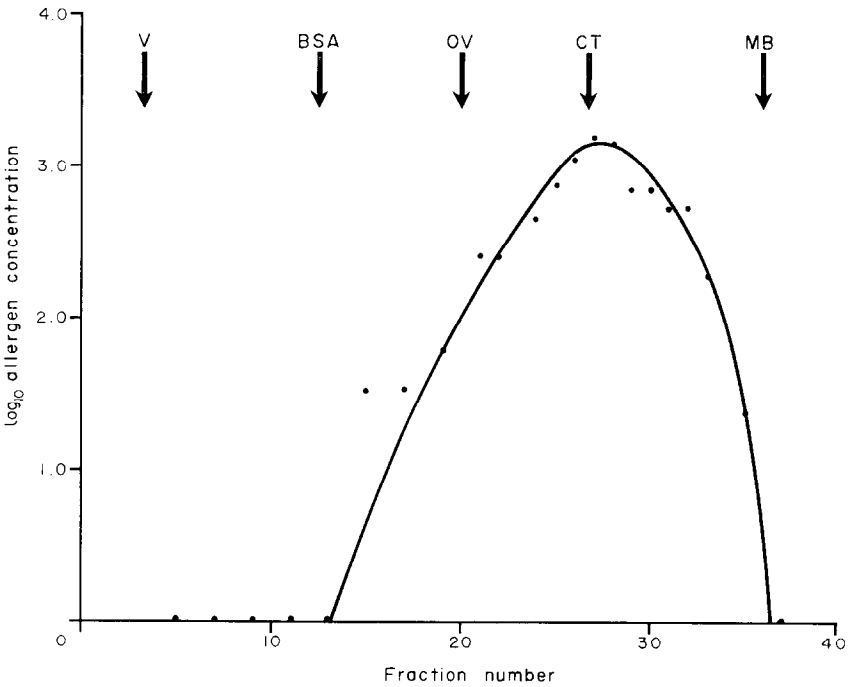

FIG. 1. Chromatography of allergen 2 on Sephadex G-100. The arrows show the elution volumes of standard proteins. V, void volume; BSA, bovine serum albumin; OV, ovalbumin; CT, chymotrypsinogen; MB, sperm whale myoglobin.

2·5 × 25 cm column of Sephadex G-100 Superfine in 0·1 M-Tris buffer pH 7·5. Also shown in Fig. 1 are the elution volumes of bovine serum albumin, ovalbumin, bovine chymotrypsinogen and sperm whale myoglobin. Using the data given by Andrews (1970), the apparent molecular weight of the allergen is 24000.

For the work to be described, it was necessary not only to partially purify allergen 2 but also to separate it from allergen 1. That this has been achieved is shown by the chromatography depicted in Fig. 1. Allergen 1, with an apparent molecular weight on Sephadex chromatography of 60,000 (Willadsen & Williams, 1976), should, if present, be eluted at fraction 10. There was negligible allergenic activity there (< 0·1% of the maximum activity).

The separation is also shown by the dermal reactions since in some animals, allergen 2 reacted much more strongly than allergen 1. This is shown for one animal in Fig. 2. The amount of allergen 1 required to give a dermal reaction is at least 300 times greater than the amount of allergen 2 necessary to give the same reaction size.

Correlation of resistance level with various immunological factors

Herd 1 contained twenty-one Herefords, purchased in south-eastern Queensland, and 12 beef Shorthorns, purchased in northern Queensland, from near Cloncurry. All animals were heifers, from 2 to 2½ years old. Serum was first collected for the indirect haemagglutination and Prausnitz–Küstner

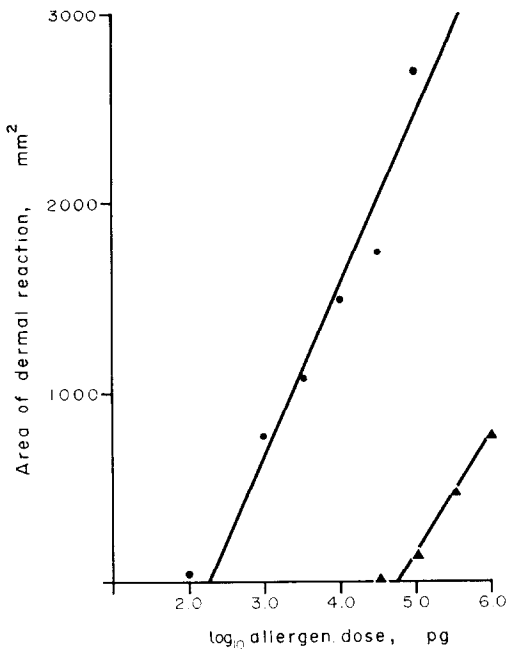

FIG. 2. Relationship between areas of dermal reactions and amounts of allergen injected. Allergen 1 (▲). Allergen 2 (●).

reactions, then the immediate hypersensitivity tests were performed. The experiment was completed within 1 week, to minimize any time-dependent changes in the animals. The results from the immediate hypersensitivity tests and haemagglutination reactions are shown in Table 2. The experimentally measured dermal reactions have been used to calculate two data points for each allergen—the amount of allergen needed to give a 500 mm² reaction, and the amount needed for a 10 mm² one. Although the areas chosen are to some extent arbitrary, the former is in the range which is most convenient and accurate to measure. Although the smaller reaction size cannot be directly measured, it represents an extrapolation to a reaction size closer to the scale of the tick feeding lesion. In almost all cases, the calculated allergen doses fell within the experimental range of 1–6 $\log_{10}$ pg. In no case were the values extrapolated more than one log unit outside this range.

To assess the relationship between allergen 1 and tick resistance, the multiple regression of $\log_{10}$ per cent tick yield on allergen 1 dose and haemagglutination reaction was calculated. Two variables were used for allergen 1, the dose required for a 10 mm² reaction and the dose required for a 500 mm² reaction. The simple correlations were both significant, ($r = 0{\cdot}76$; $P < 0{\cdot}001$ for 10 mm² reaction and $r = 0{\cdot}43$, $P < 0{\cdot}01$ for 500 mm² reaction). The multiple regression coefficient for 10 mm² reaction was highly significant ($b = 0{\cdot}38$; $f = 5{\cdot}97$, $P < 0{\cdot}001$) but that for the 500 mm² reaction was not ($b = 0{\cdot}03, f = 0{\cdot}40$).

The haemagglutination data did not correlate significantly with log tick yield alone ($r = 0{\cdot}32$) but the multiple regression coefficient was significant after allowing for the allergen 1 response ($t = 3{\cdot}25$, $P < 0{\cdot}01$). There was no indication of an interaction between responses to allergen 1 and the haemagglutination titres.

Figure 3 shows the log % tick yields plotted against the calculated allergen 1 doses. The raw data, not corrected for haemagglutination titre, were used.

The data for allergen 2 were treated similarly, using again the allergen dose required for 10 mm² and 500 mm² reactions. Both simple correlations were significant ($r = 0{\cdot}53$, $P < 0{\cdot}01$ for the 10 mm² reactions and $r = 0{\cdot}62$, $P < 0{\cdot}01$ for the 500 mm² reactions). The multiple regression coefficients were both similar ($b = 0{\cdot}139$, $t = 1{\cdot}94$, $P < 0{\cdot}10$ for 10 mm² and $b = 0{\cdot}133$, $t = 2{\cdot}74$, $P < 0{\cdot}02$ for 500 mm² reaction). The response at 250 mm² was estimated to give maximum correlation.

There were variations in the sizes of the Prausnitz–Küstner reactions from a single test serum injected into different recipient calves. That this was due to differences in the recipients was shown by the fact that duplicate injections into different skin areas of a single calf gave reproducible results. Because of this variability, only one allergen was tested in any particular calf and the 16 or 17 test sera per calf were chosen to cover the full range of resistance levels. These results were then considered as a single group in attempting to correlate tick yield with the Prausnitz–Küstner reactions. Both tick yields and log tick yields were correlated with the reaction sizes at each serum dilution and with the estimated endpoint dilution for each serum. None of the correlations was significant for either allergen.

For the interpretation of these results, it is important to know that the reactions reflect specific immunological responses and not non-specific factors. Injection of 0·5 μg of either allergen 1 or allergen 2 into 12 unexposed animals did not give a reaction greater than a saline control in any of them, whereas all exposed animals tested reacted to this amount of allergen.

The haemagglutination reaction was tested using eleven sera from unexposed cattle. Ten of the eleven gave zero titres and one gave a titre of 6. The latter figure could arise from some unknown immunological cross-reaction or from an earlier accidental exposure to ticks. The result shows that non-specific reactions should be uncommon. Five sera from unexposed animals were included in the Prausnitz–Küstner tests for both allergens but none gave reactions greater than the saline controls. Thus the results obtained in the field tests may be assumed to reflect immunolgoical responses to tick exposure.

TABLE 2—CORRELATIONS OF TICK YIELD WITH RESPONSES TO ALLERGENS 1 AND 2 AND INDIRECT HAEMAGGLUTINATION TITRES

Animal	Tick yield (%)	Allergen 1 dose (log_{10}pg)		Allergen 2 dose (log_{10}pg)		Haemagglutination titre
		10 mm²	500 mm²	10 mm²	500 mm²	
1	0·4	2·5	4·8	0·1	2·2	7
2	0·5	1·6	3·0	1·1	2·5	2
3	0·9	1·5	4·0	0	1·3	7
4	1·0	1·7	4·1	1·6	2·8	5
5	1·7	4·6	5·6	2·2	2·8	0
6	2·2	2·8	4·9	1·8	4·4	1
7	2·3	3·4	4·3	1·6	2·8	2
8	2·4	3·8	6·5	2·4	5·4	6·5
9	2·4	4·0	5·1	2·2	3·4	0
10	3·0	4·2	5·4	1·3	3·6	7
11	3·3	2·9	3·6	2·6	3·0	6
12	3·6	3·8	4·8	0·5	2·1	6
13	3·8	3·7	4·5	2·2	2·6	4·5
14	4·0	3·9	5·2	2·2	3·1	0
15	4·4	3·5	6·8	1·4	5·0	6·5
16	5·3	4·0	4·8	1·3	3·3	6
17	5·6	4·3	5·5	0·5	6·0	6
18	5·7	3·5	4·1	0·7	3·3	7·5
19	5·7	4·1	4·8	1·3	3·2	1·5
20	6·2	3·5	5·1	2·7	4·4	8
21	7·8	4·0	5·6	3·0	5·7	5
22	8·0	4·1	5·3	4·1	5·4	0
23	8·3	4·5	6·1	2·3	5·2	6
24	8·6	4·4	5·1	3·4	4·9	8
25	8·6	3·0	6·7	5·0	—	8·5
26	9·2	4·1	5·1	1·6	3·1	4
27	11·4	3·7	4·3	1·5	2·9	7·5
28	11·7	4·0	5·7	6·0	—	9·5
29	13·0	4·1	4·7	3·4	3·9	3
30	13·7	4·5	5·8	1·9	4·6	
31	14·1	4·9	5·9	2·7	3·7	4
32	15·7	4·5	5·6	2·1	7·0	5
33	18·1	5·0	6·1	2·9	7·0	5·5

Immediate hypersensitivity tests in other herds

Herd 2 contained 16 AIS cows, with ages ranging from 3 to 6 years. They were from an inbred herd of animals which, although it had been selected for tick resistance, still contained animals with a wide range of resistance levels. Herd 3 contained 22 Hereford–Shorthorn crossbred cattle and 21 Afrikander Crossbreds. Both herds were tested with allergen 1 exactly as described for herd 1. The tick numbers used in calculating correlations were from natural infestations as described under Methods. With both herds, there was no significant correlation between tick numbers and the allergen 1 dose for 500 mm² reactions ($P > 0{\cdot}1$). However, the correlations were highly significant with the data calculated for 10 mm² reactions ($P < 0{\cdot}001$).

DISCUSSION

The cattle in this study had no contact with antigens from *B. microplus* other than by infestation, so the immune reactions described must have arisen naturally as a result of the host-parasite interaction. There is a highly significant correlation between the tick yield and the intensity of the immediate hypersensitivity reaction by the host to two tick allergens. Hosts of high resistance reacted to smaller amounts of allergen than did hosts of low resistance. The importance of sensitivity to small quantities of allergen may simply be a reflection of the small quantities introduced by larvae. For example, a simple calculation shows that the amount of allergen 1 contained in a single, unfed larva will be between 1 and 10 ng, while the amounts of allergen 1 listed in Table 2 which correlate well with tick yield range from 0·01 to 100 ng. These two extremes may reflect on one hand a highly resistant host which will respond immunologically to a larva as soon as it starts injecting allergen and, on the other hand, a host of low resistance which will respond little, if at all, to the amount of allergen which a larva could be expected to inject.

Even if it is assumed that there is a causal relationship between immediate hypersensitivity and resistance, there are numerous possible explanations. The two allergens could be critical for the success of

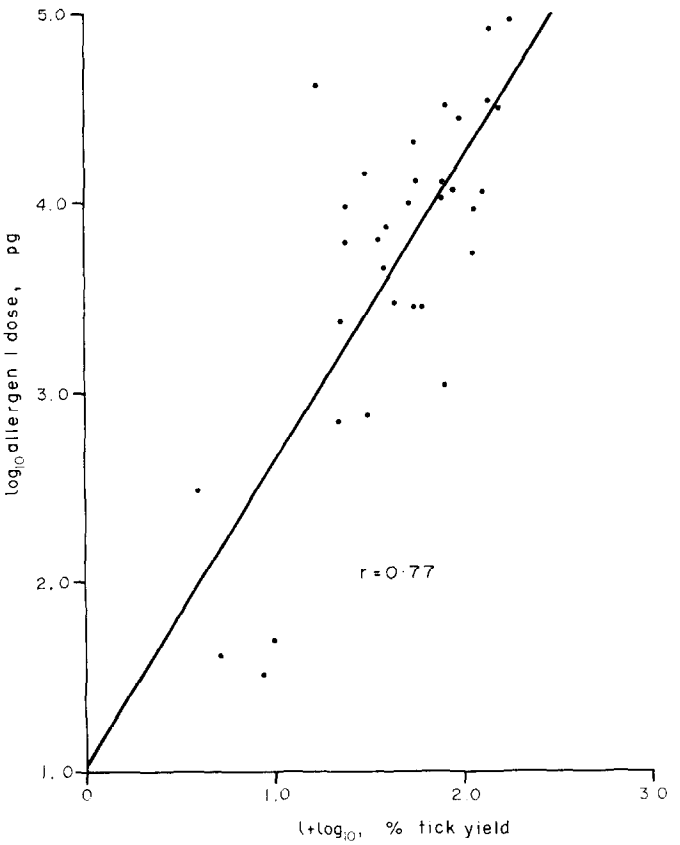

FIG. 3. Correlation between the calculated amount of allergen 1 giving a dermal reaction 10 mm² in area and the logarithm of the % tick yield.

the tick and the hypersensitivity reaction might interfere with their functions. Allergen 1, which is an esterase, may be the enzyme observed histologically shortly after the first attachment of the tick (Schleger & Lincoln, 1976). Alternatively, it might be that particular hosts are more resistant because they produce more of, or are more sensitive to, some of the pharmacologically active compounds released during the immediate hypersensitivity reaction. The present study eliminates only one of many possibilities, that is, that the important factor is the availability in serum of specific IgE. There was no correlation between the Prausnitz–Küstner reactions and either the resistance levels of the donors of the serum or the active reactions of those donors. This is in contrast to experience in human medicine, where there are numerous examples of correlations between specific IgE levels in serum, active skin reactions and clinical allergy (Chua, Bremner, Lakdawalla, Llobet, Kokubu, Orange & Collins-Williams, 1976; Miyamoto, Johansson, Ito & Horiuchi, 1974).

Little is known about the ways in which pharmacologically active compounds are involved in resistance. However, histamine is released by mast cells in immediate hypersensitivity reactions in bovines (Wells & Eyre, 1972) and its presence beneath the attachment sites of young larvae causes them to detach (Kemp, 1977). As well, an eosinophil-chemotactic factor is released by degranulating mast cells (Goetzl, Wasserman & Austen, 1975). The eosinophils of a highly resistant host are more strongly attracted to the immediate vicinity of the tick's mouthparts, and degranulate more, than do the eosinophils of a host of low resistance (Schleger, Lincoln, McKenna, Kemp & Roberts, 1976).

The correlation between tick yield and the haemagglutination titres is much weaker than the correlations already discussed and the mode of action of the responsible antibody is unknown. An agglutinating antibody could correlate with resistance by acting as a blocking antibody towards allergen 1. However, in this case, it would be reasonable to expect a negative correlation between allergen 1 reactions and haemagglutination titres. This was not observed, which suggests that the antibody, or

an antigen–antibody complex formed at the tick attachment site, might play some unknown role distinct from the hypersensitivity reaction.

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