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Boophilus microplus: Cellular Responses to Larval Attachment and Their Relationship to Host Resistance

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Abstract

The histology of early feeding lesions of the cattle tick *B. microplus* has been studied using ^{32}P labelled larvae to standardize the duration of attachment. Critical studies were made on 3-h lesions in six separate experiments on different groups of British breed animals. Each group consisted of three animals—one previously unexposed to ticks, one of high resistance and one of low resistance.

The degree of mast cell disruption, eosinophil concentration and degranulation, and the extent of epidermal vesiculation were all significantly greater at the site of attachment on highly resistant hosts. In previously unexposed animals there was no mobilization of eosinophils nor mast cell breakdown and no epidermal vesiculation.

Possible immune mechanisms producing mast cell disruption and the infiltration and concentration of eosinophils are suggested, and the effect of eosinophil degranulation on larval attachment and feeding is discussed.

Introduction

Cattle show differences in their resistance to infestation with the cattle tick *Boophilus microplus* (Wilkinson 1955). Resistance is immunologically mediated (Roberts 1968*a*) and the potential of the host to acquire a particular degree of resistance is heritable (Wharton *et al.* 1970). In European breed cattle, resistance affects the establishment of each instar of this one-host tick, and it is particularly manifest against larvae in the first 24 h of their parasitic life (Roberts 1968*b*).

Tatchell and Moorhouse (1968) and Tatchell (1969) studied the histological changes of the skin associated with attachment and feeding of all stages of *B. microplus* on *Bos taurus* and *B. indicus* cattle. They compared animals with and without tick experience, and investigated the lesions in animals having different levels of resistance. Larval attachment (up to 5 h) on previously exposed *B. taurus* was characterized by dilation of dermal capillaries and an infiltration and disruption of eosinophils. Cellular infiltration was related to the acquisition of resistance, in that it was at a very low level in primary infestation but there appeared to be no relationship between its intensity and the degree of resistance of the host. It was concluded that the dermal eosinophilic response in British breed cattle contributed to their susceptibility to infestation. However, in Zebu cattle there appeared to be a relationship between infiltration of the epidermis and the degree of resistance.

Subsequently it was found that early attachments of larvae are not stable (Roberts 1971), so that if a relationship exists between cellular reaction and resistance level of

the host it may have been confounded in the study of Moorhouse and Tatchell (1968) by lack of knowledge of the duration of the attachments. Kemp *et al.* (1971) successfully labelled *B. microplus* larvae with ^{32}P and so were able to monitor a continuous attachment on the host and determine its duration.

In the present study, ^{32}P labelled larvae were used for observing the cellular infiltration at the attachment site during the first 3 h of the parasitic life cycle. This time is critical because it approaches the mean duration of the first attachment on highly resistant animals (Kemp *et al.* 1976) and is also optimal for measuring the degree of eosinophilotaxis (see p. 502).

Materials and Methods

Experimentation

Over a period of 12 months, six separate experiments were performed on different groups of animals, each group consisting of three animals—one animal which had not been previously exposed to *B. microplus*, one animal of low resistance, and one animal of high resistance. The unexposed animal in each experimental group was younger than the previously exposed animals, but older animals are equally unresponsive to primary infestation.

Animals

All experimental cattle were of British breed, predominantly Australian Illawarra Shorthorn. The unexposed cattle were obtained from an area where *B. microplus* does not occur.

Infestation

The cattle were held in covered pens and infested daily with 1000 larvae as described by Roberts (1968*b*). Their resistance was measured by counting the number of female ticks engorging. Those yielding 0–2% of the potential yield of adult females were regarded as highly resistant, and those yielding 10% or more as of low resistance.

Larvae

Larvae were labelled with approximately 5 nCi of ^{32}P (Kemp *et al.* 1971), after which a sample of 50 was placed on the neck in an area favoured by the tick, and on the flank, an unfavoured area. Thirty minutes later the larvae were located using a Geiger–Muller counter (Geiger–Muller counter type LB 1200, Berthold Ltd) with an end window diameter of 28 mm as described by Kemp *et al.* (1976). Approximately 40–60% of the larvae were found attached in the two infested areas. Attachment sites were marked with a spot of white paint on the adjacent hair and relocated at intervals to ensure that the larvae had not moved. At the appropriate time the attachment area was infiltrated with 2·0 ml of 2% xylocain (Astra Chemicals Pty Ltd, North Ryde, N.S.W.) and a skin biopsy was taken with a 0·5-cm diameter trephine. Biopsies with attached larvae were taken from both the neck and flank areas for each of two methods of embedding.

Histology

For paraffin embedding the biopsies were trimmed to within 2 mm of the mouthparts of the larva, dipped in fresh egg albumin and fixed in 10% alcoholic formalin (Lillie 1965) for 48 h. The biopsies were transferred to ascending grades of alcohol, xylol and embedded in paraffin. Sections 8 μm thick were cut on a rotary microtome. Segments of the paraffin ribbon were examined under a dissection microscope and 10–15 sections through the attachment site were retained for staining with chromotrope-2R Leishman (Anon. 1966).

Biopsies intended for epoxy embedding were trimmed to approximately a 2 mm cube while fixing in formaldehyde-glutaraldehyde solution (Karnovsky 1965) and post-fixed in alcoholic formalin. The fixed tissue was processed through ethanol and propyleneoxide, and blocked in low viscosity embedding medium (Spurr 1969). Sections 0·5 μm thick were cut on a Porter Blum MTI microtome and stained with 0·25% alcoholic eosin followed by an azur II–methylene blue mixture. Details of the method will be described elsewhere (R. U. McKenna, unpublished data).

An eyepiece graticule of 100 squares was used in assessing the sections at a magnification of $\times 400$ for the 8-μm sections and $\times 630$ for the 0·5-μm sections. In the 8-μm sections the inflammatory area was generally divided into a central portion of eosinophil concentration and an ill-defined peripheral portion where the eosinophils were relatively sparse. The central area was measured by the number of squares, each square representing a sectional area of $6{\cdot}5 \times 10^{-4}$ mm^2. Four fields of the lesion were studied in the 0·5-μm sections—the most central field adjacent to the mouthparts, two fields on either side, and the field immediately below. Each field represented an area of $2{\cdot}7 \times 10^{-2}$ mm^2 and each individual square an area of $2{\cdot}7 \times 10^{-4}$ mm^2. Similar fields were studied in a control area, intermediate between the lesion and the edge of the section.

The 0·5-μm sections were mounted two per slide and each fifth slide was examined for eosinophil granules and for mast cells. These assessments were limited to two and three experiments respectively.

In both the 8-μm and 0·5-μm sections the eosinophils in each square of the graticule were assessed for degranulation and counted. In the 0·5-μm sections a cluster of intracytoplasmic granules indicated an intact eosinophil and the granules could be classified in all squares. Intact eosinophils in the 8-μm sections were readily identifiable but, due to the concentration of cells and granules in the central inflammatory area of some previously exposed animals, assessments were made in the peripheral areas only. The degree of degranulation in lesions of high-resistance animals, due to their greater cellular concentration, tended therefore to be underestimated.

Mast cells could not be distinguished in some 8-μm sections due to the dense accumulation of eosinophils. In the 0·5-μm sections identifiable mast cells were scored from 1 to 7, according to their granular content as follows: 1, packed with granules; 2, 3, 4, granules of medium, sparse and very sparse distribution respectively; 5, granules distributed peripherally; 6, 7, two degrees of granular exocytosis.

Results

Choice of Attachment Time

In preliminary experiments, attachments of 1, 3 and 5 h duration were obtained from each of three animals. In the previously exposed animals the cellular infiltration was much greater and less variable at 3 h than at 1 h. On the other hand, in most high-resistance animals the 5-h attachments contained fewer dermal cells than the 3-h attachments, the infiltrating cells having invaded the epidermis and formed a vesicle. Consequently, detailed studies were confined to 3-h attachments. The choice of a 3-h attachment time, however, may not have been optimal for all experiments. In a number of 3-h biopsies, especially from the favoured area of high-resistance animals, cellular infiltration could not be quantitated as it had already reached the stage of invasion and vesiculation of the epidermis.

Biopsies were restricted to a 5-h attachment time in the previously unexposed animals to provide a greater time for infiltration of eosinophils.

Mast Cell Changes

The mean number of mast cells within the defined area of the lesion, and within a 'control' area from the same biopsy, are set out in Table 1 for three experiments. In the previously exposed animals there was a reduction in mast cell number near the larval lesion. Free and phagocytosed mast cell granules indicated cell breakdown. In the high-resistance animals, the mean reduction was 60·7% in favoured areas and 58·2% in unfavoured areas, whereas in the low-resistance animals the reduction was 50·1 and 15·7% respectively. Analysis of variance, using log transformation and mean values weighted for number of sections examined, showed a significant difference between resistance levels and also between favoured and unfavoured areas. There was no decrease in the number of mast cells near the attachment site in previously unexposed

animals. The mast cells in the dermal lymphoid tissue of a control area and of an area near a 1-h attachment site are shown in Figs 1 and 2, respectively, for a high-resistance animal.

The overall mean percentage of mast cells showing a high degree of degranulation (score >4) is included in Table 1. Whereas the reduction in number of detectable mast cells in the central area of the lesion was greater in the high-resistance animals, the degree of degranulation of the identifiable mast cells in these animals appeared lower. The differences, however, were not statistically significant. Although there was no reduction in number of mast cells in lesions from unexposed animals, the degree of degranulation was comparable with that in low-resistance animals. The degree of mast cell degranulation in the 'control' area of the unexposed animals, relatively close to the lesion, was greater than that normally found in a biopsy free of larvae.

Table 1. Number of dermal mast cells within $1{\cdot}08\times10^{-1}$ mm of six 3-h larval attachment sites, and within a comparable subepidermal area from the same biopsy, for each of three experimental groups of animals, and the proportion of mast cells showing a high degree of degranulation

The number of mast cells is adjusted to the mean number (10) of 0·5-μm sections examined per lesion

Animal	Area		18.vi.73	20.vii.73	4.x.73	Percentage degranulation
High resistance	Favoured	Lesion	26	26	45	40·2
		Control	57	81	112	40·7
	Unfavoured	Lesion	16	19	14	46·8
		Control	40	31	58	39·6
Low resistance	Favoured	Lesion	43	17	59	57·7
		Control	96	47	86	60·0
	Unfavoured	Lesion	—	70	70	72·3
		Control	—	68	83	55·6
Unexposed[A]	Favoured	Lesion	70	146	—	70·1
		Control	64	127	—	51·5
	Unfavoured	Lesion	111	50	63	56·8
		Control	46	43	83	54·5

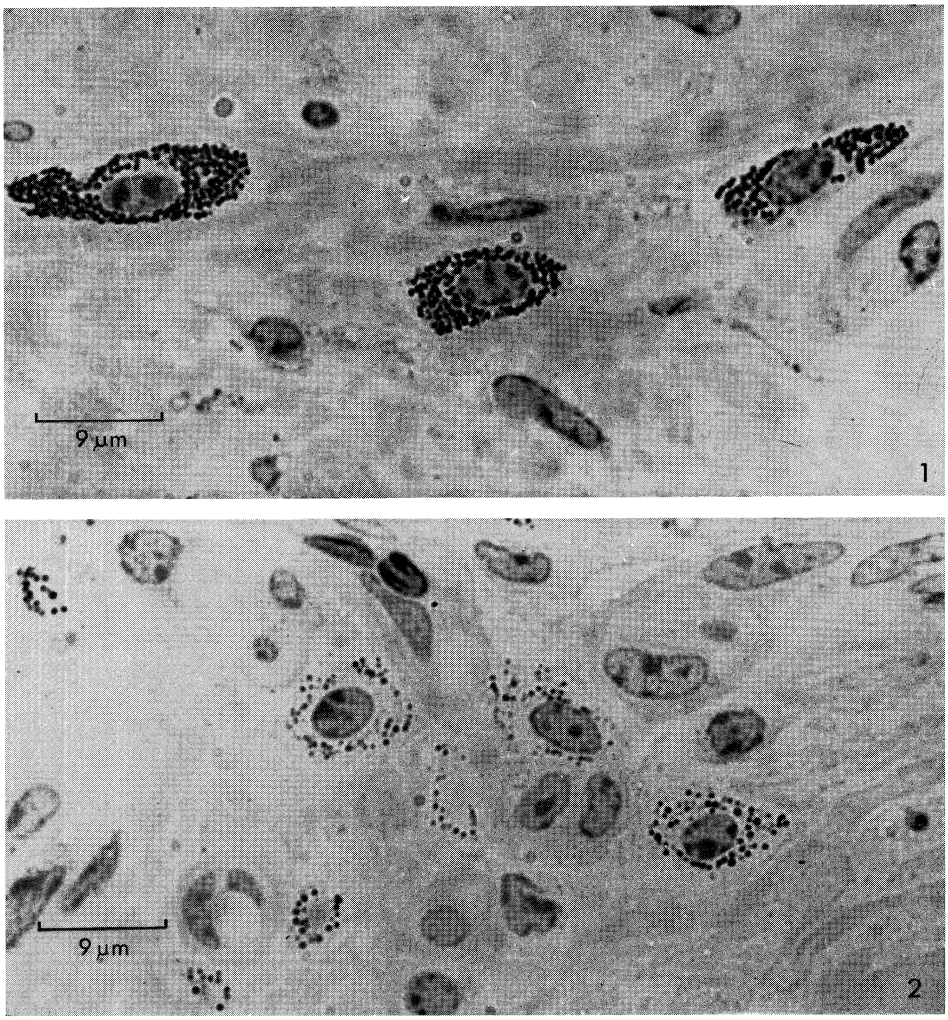
[A] 5-h lesion.

Differences in Tissue Eosinophilia

Virtually no eosinophil infiltration was observed in the lesions of previously unexposed animals (Fig. 3), although the attachments were of longer duration. Occasionally a degranulated eosinophil or a few free eosinophil granules were observed in the vicinity of the epidermal basement membrane near the attachment site. When lymphatic tissue occurred near the attachment site, the number of eosinophils was comparable with that in uninfested tissue, although there might be a slight degree of degranulation.

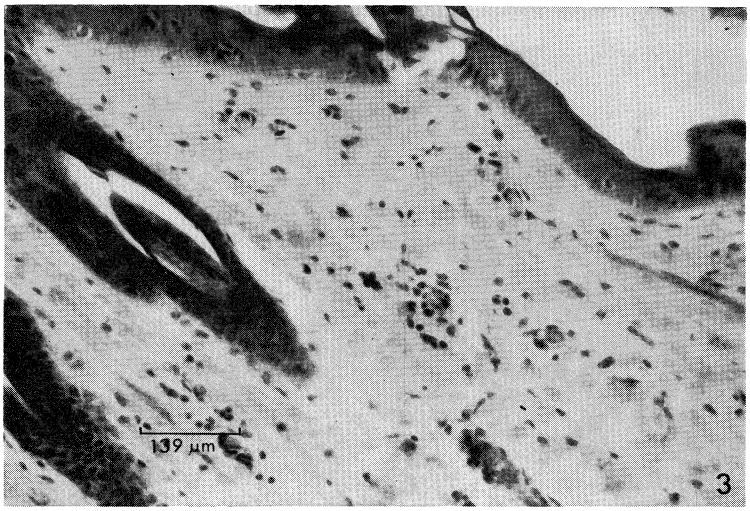
In the 3-h lesions of low-resistance animals (Fig. 4) eosinophil concentration was at a relatively low level. A large number of intact eosinophils was observed intra-

vascularly. Those which had infiltrated the dermis showed little degranulation and little or no attraction to the attachment site. The central area of concentration and degranulation was usually poorly defined.



Figs 1 and 2. Dermal mast cells in a high-resistance animal which are packed with granules (Fig. 1) and those adjacent to a 1-h attachment site which contain few granules (Fig. 2). Epon sections, 0·5 µm thick, stained with 0·25% alcoholic eosin and azur II–methylene blue.

Eosinophils predominated in the cellular infiltration of previously exposed animals at 3 h. In the high-resistance animals (Fig. 5), the concentration of eosinophils at the attachment site and their degree of degranulation were greater than in the low-resistance animals. Invasion of the epidermis had commenced in some 3-h lesions from high-resistance animals and discrete vesicles had formed in others (Figs 8 and 9).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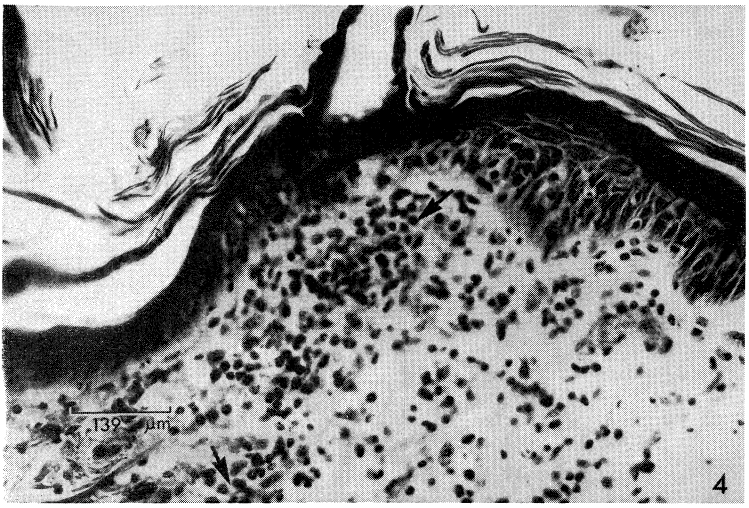
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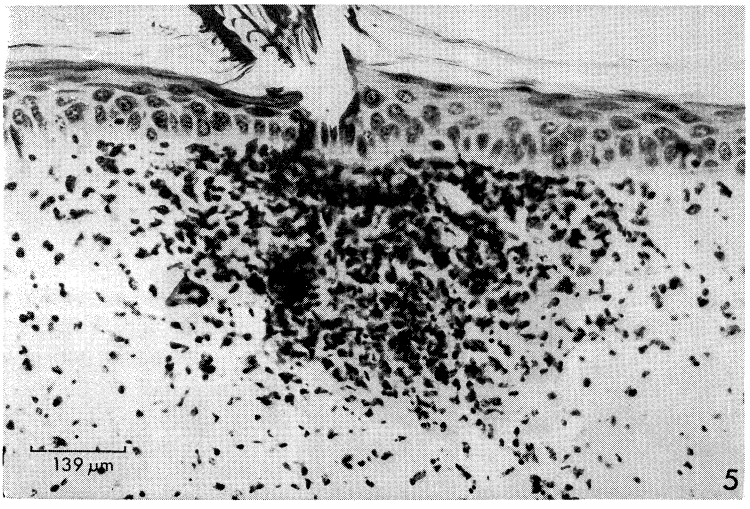
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Eosinophil degranulation was more clearly observed in the 0·5-μm epon sections as shown in Fig. 6.

Degree of Eosinophil Infiltration, Concentration and Degranulation

Five parameters were used to assess the extent of eosinophil mobilization: (1) central area of eosinophil concentration, (2) total number of eosinophils in the central area, (3) concentration of eosinophils, i.e. ratio of eosinophil count in central and peripheral areas, (4) total numbers of eosinophils in the central and peripheral areas and (5) percentage degranulation of eosinophils.

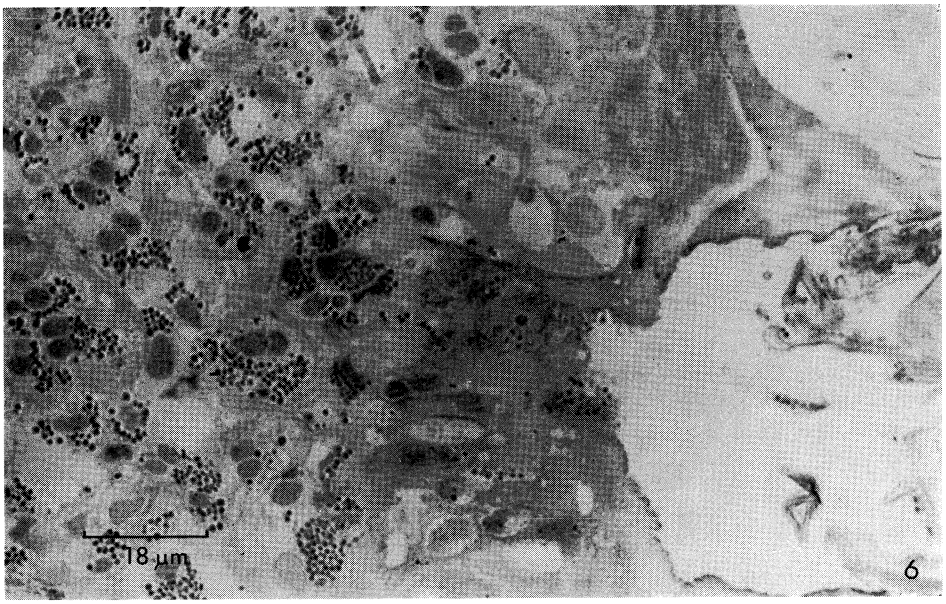


Fig. 6. Epon sections showing eosinophil degranulation in the 3-h larval lesion of a high-resistance animal. Alcoholic eosin (0·25%) and azur II–methylene blue stain.

The mean value for each parameter, derived from three sections per lesion over the six experimental periods, is set out in Table 2 for each area and level of resistance. The total number of lesions involved in each set of parameters is indicated. The analysis of variance on the transformed cell means is set out in Table 3; the results are substantially the same as those on the untransformed data. There were highly significant differences between resistance levels in area of central eosinophil concentration, eosinophil count in the central area, and central/peripheral ratio of eosinophil count. The extent of eosinophil concentration in the low-resistance animals was not only less, but less variable also. There was little variation between

Figs 3–5. Differences in eosinophilotaxis shown by a comparison of 3-h larval lesions in a previously unexposed animal (Fig. 3), a low-resistance animal (Fig. 4), and a high-resistance animal (Fig. 5). There is no cellular infiltration in Fig. 3. In Fig. 4 the eosinophils are largely intact and the intravascular location of some is indicated by their linear arrangement (arrows). In Fig. 5 the eosinophils are concentrated at the basement membrane adjacent to the larval mouthparts. Chromotrope–Leishman stain.

the two areas of these animals, and little difference between the low-resistance animals sampled on different dates. The percentage degranulation of eosinophils, which could

Table 2. Means of five parameters denoting degree of eosinophil infiltration, concentration and degranulation in 3-h larval lesions from animals of high and low tick resistance

Means are derived from three 8-μm sections from each lesion obtained in six different experiments. N_c, Number of eosinophils in central area; N_p, number of eosinophils in peripheral area; percentage degranulation values are for the peripheral area

Animal	Area	No. of lesions	$10^4 \times$ Central area of concn (mm^2)	N_c	N_c/N_p	N_c+N_p	% Degranulation
High resistance	Favoured	14	122·9	181	5·8	237	38·2
	Unfavoured	12	102·1	106	6·5	148	39·7
Low resistance	Favoured	11	35·8	52	0·8	162	19·8
	Unfavoured	12	26·7	37	0·6	104	20·4

be assessed only in the less dense peripheral inflammatory area in 8-μm sections, was significantly greater in highly resistant animals. The total eosinophil count did not differ significantly for the two levels of resistance.

Table 3. Analysis of variance of parameters representing degree of eosinophil infiltration, concentration and degranulation in 3-h larval lesions

Mean values for the parameters are shown in Table 2. Error is the between-animal variation. s.s., Sum of squares; F/e.m.s., F ratio/error mean squares

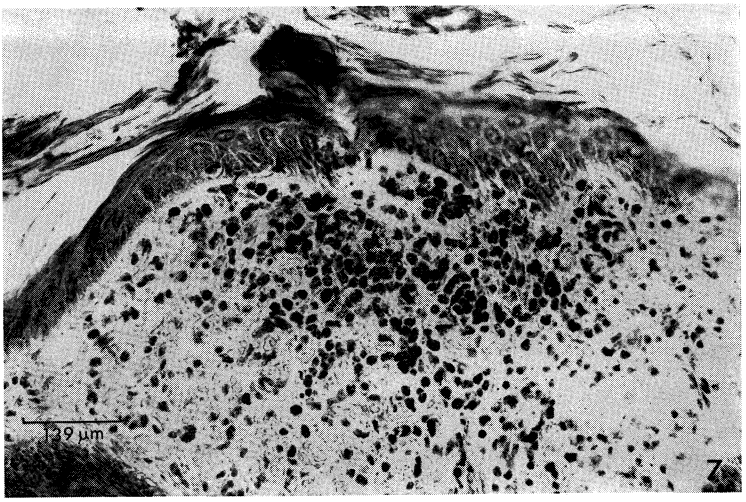
Transformed source	d.f.	Analysis of variance	Central area	N_c	N_c/N_p	N_c+N_p	% Degranulation
Resistance	1	s.s.	60027	99670	223022	17986	44452
		F/e.m.s.	22·82**	13·47**	19·66**	3·78	12·44*
Experiments	5	s.s.	57830	137182	29868	78227	58466
		F/e.m.s.	4·40	3·71	2·63	3·29	3·27
Error	5	s.s.	13148	22682	13371	41031	17871
		F/e.m.s.	2630	(4536)[A]	(2674)[A]	8206	3574

* $P < 0·05$. ** $P < 0·01$.

[A] These error mean squares were not used because the within-animal variation was greater.

When the data from favoured and unfavoured areas were analysed separately there was a tendency for the differences between resistance levels to be greater in the favoured areas, although this was not statistically significant. The effect is reflected

Figs 7–9. Two types of epidermal vesicles (Figs 7 and 8) in 3-h larval lesions in the favoured area of high-resistance animals. In Fig. 7 the small, well-defined vesicle (arrow) is on the edge of a hair follicle infundibulum. A number of relatively intact eosinophils are contained therein. The vesicle in Fig. 8 is rather complex, consisting of a series of discrete vesicles, each containing infiltrated eosinophils. The larger vesicles (arrows) are contained in the adjacent follicle infundibula. In the more advanced 5-h lesion of a high-resistance animal (Fig. 9) the discrete vesicles have disappeared and one large bleb has formed. Chromotrope–Leishman stain.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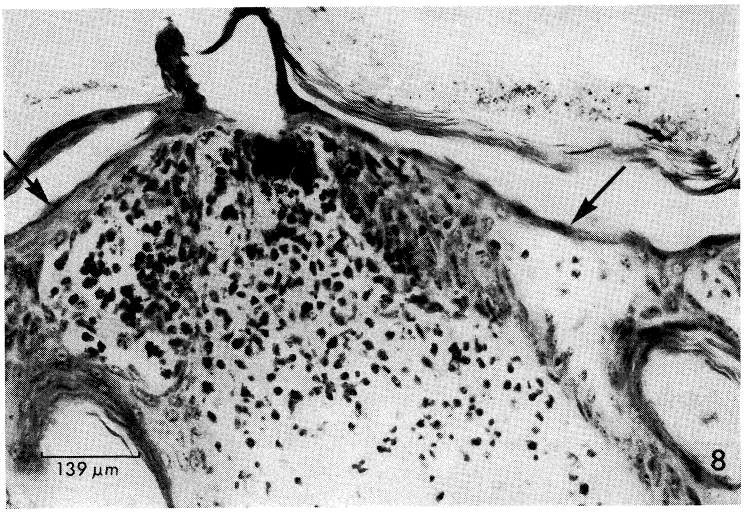
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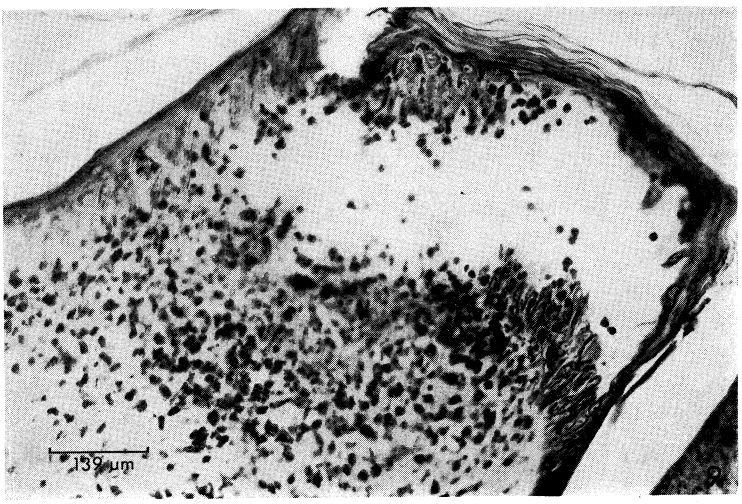
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in Table 4 in a comparison of F ratios and estimates of the difference for the five parameters analysed in Table 3. As indicated below, the degree of epidermal vesiculation was greater in the favoured areas, especially in animals of high resistance.

In the 0·5-μm sections intracytoplasmic eosinophil granules appeared in clusters bounded by the cell membrane, and could be distinguished from free granules. The mean counts of eosinophil granules, both free and intracytoplasmic, are set out in Table 5 for the high- and low-resistance animals from two experiments.

Table 4. Comparison between favoured and unfavoured areas of the difference between high- and low-resistance animals in the five parameters listed in Table 2, using F-ratios and estimates of the difference in the transformed scale

	Area	Central area	N_c	N_c/N_p	N_c+N_p	% Degranulation
F-ratio	Favoured	23·87	17·99	14·74	2·30	5·91
	Unfavoured	4·97	2·55	5·64	0·36	4·70
Estimated difference	Favoured	0·39	0·50	0·63	0·23	0·28
	Unfavoured	0·24	0·31	0·59	0·12	0·25

The ratio of free/intracytoplasmic granules was used as an index of degranulation and its variance was analysed. A log transformation was used to approximately normalize the variation. A one-way analysis of variance showed significant differences and also heterogeneity of variation despite transformation. An analysis of means was made, weighting each value by the inverse of its estimated variance. Despite the small number of observations (eight), differences between resistance levels and

Table 5. Number of eosinophil granules, both free and intracytoplasmic, the ratio of the granules of each type, and the number of intracytoplasmic clusters (N) in the 3-h larval lesions for two experiments

The number of eosinophil granules is adjusted to the mean number (10) of 0·5-μm sections examined per lesion. i.c., Intracytoplasmic

Animal	Area	20.vii.73				4.x.73			
		Free	i.c.	Free/i.c.	N	Free	i.c.	Free/i.c.	N
High resistance	Favoured	1057	286	3·7	22	296	77	3·8	6
	Unfavoured	508	284	1·8	23	955	815	1·2	65
Low resistance	Favoured	1408	1757	0·8	119	148	249	0·6	21
	Unfavoured	525	525	1·0	40	424	566	0·8	42

between areas were both highly significant. The estimated mean values, using log transformations, were as follows:

	High	Low	S.e. of difference
Favoured	0·526	−0·144	0·050
Unfavoured	0·144	−0·147	0·048

The ratio of free/intracytoplasmic granules was greater for the central field than for the central and peripheral fields combined. There was no obvious difference between resistance levels in this regard and a special analysis for the central field was not carried out.

Epidermal Vesiculation

Dermal infiltration by eosinophils was followed by an invasion of the epidermis and the formation of one or more discrete vesicles (Figs 7 and 8). There was a difference between resistance levels, and between body regions, in the incidence of vesicles in the 3-h lesions. No epidermal vesicles were found in unfavoured areas. In the favoured areas, 6 out of 13 lesions had epidermal vesicles in the high-resistance animals, while only 1 vesicle occurred in 11 lesions from the low-resistance animals. Of the 7 lesions with epidermal vesicles, 5 were from experiments carried out in summer months.

In 5-h lesions, particularly in the favoured areas of high-resistance animals, the discrete vesicles had largely coalesced to form one large intra-epidermal bleb (Fig. 9). There was still a low proportion of vesicles in the lesions from the unfavoured areas, no vesicles in 7 lesions in the low-resistance animals, and 2 vesicles in 6 lesions in the high-resistance animals. In the favoured areas, each of the resistance groups had vesicles in 4 out of 6 lesions, but the vesicles in the high-resistance animals were, on the average, more than twice the size of those in the low-resistance group.

Neutrophils

Neutrophils were observed within the inflammatory area occasionally in the 3-h lesions and quite frequently in the 5-h lesions. They appeared in greater numbers in the high-resistance animals but were not quantitated.

Basophils

Basophils were occasionally observed within the inflammatory area in the 0·5-μm sections. Their numbers were insufficient to warrant quantitation.

Discussion

Studies of the histology of the attachment sites of larvae of *B. microplus* after 3 h have revealed differences between animals which are related to their levels of resistance to the tick. The degree of eosinophil concentration and degranulation at the site of attachment, the extent of mast cell disruption, and the incidence of epidermal vesiculation were all significantly greater on hosts of high resistance than on those of low resistance. In areas favoured by the tick the cellular response is more marked and is more closely related to an animal's resistance level than in an area not favoured by the tick. Previously unexposed animals showed no mobilization of eosinophils nor mast cell disruption, and no epidermal vesiculation.

Degranulation of mast cells is an important event in the initiation of an immediate immune response (Keller and Schauwecker 1972). Vasoactive amines, heparin and related mucopolysaccharides, and eosinophil chemotactic factor of anaphylaxis (ECF-A) are all rapidly released (Selye 1965; Ishizaka *et al.* 1972; Kaliner *et al.* 1972; Wasserman *et al.* 1974). A major mechanism of release is via the reaction of antigen with specific reaginic antibody on the surface of the mast cell (Parish 1972), but release related to complement fixation also occurs (Movat 1971). Degranulation may occur by release of active constituents from granules *in situ*, or there may be severe disruption of the cytoplasm and shedding of granules (Selye 1965). Biologic polymers which can degranulate mast cells occur in insect saliva (Johnson and Erdos 1973) and

venoms (Habermann 1972) and there is a degranulating agent in *Nippostrongylus braziliensis* (Uvnäs and Wold 1967). While there was no mast cell destruction in previously unexposed animals, a low degree of degranulation did occur. This could result from a degranulating agent in the saliva of *B. microplus* which increases permeability of capillaries as has been observed following injection of adult saliva into unexposed bovine hosts (Tatchell and Binnington 1973).

In the immediate vicinity of a larval attachment site, mast cell breakdown was greatest in hosts of high resistance. Whilst the destruction of cells was much less in low-resistance hosts, mast cells over a wide area showed some degranulation which suggests a wider diffusion of salivary antigen in such cases. Local degranulation of mast cells occurs during the passage of *N. braziliensis* through the lungs of the rat in the initial phase of infection (Wells 1971), and in the small intestine of the guinea-pig after a challenge infection with *Trichostrongylus colubriformis* (Rothwell and Dineen 1972). In acute inflammatory conditions in the skin (Mikhail and Miller-Milinska 1964) and gingiva (Zachrisson 1969) of man, mast cell degranulation occurs.

As well as ECF-A from mast cells, there are immunologically derived chemotactic factors which could contribute to the attraction of eosinophils to the attachment site. These factors are produced by antigen–antibody complexes, complement fixation and lymphocyte stimulation (Higashi and Chowdhury 1970; Kay 1970; Cohen and Ward 1971; Colley 1973). The chemotactic factors released from mast cells, and those associated with lymphokines, are exclusively eosinophilotactic in contrast to the factors produced by fixation of complement, which include an attractant for neutrophils. At 3 h the polymorphonuclear leucocytes in the tick lesions were predominantly eosinophils which may indicate that complement fixation is not important at that stage. In the dog, attachment by the adult female tick *Rhipicephalus sanguineus* produced no appreciable dermal infiltration within the first 24 h, after which time mast cell degranulation occurred and there was an infiltration of neutrophils (Theis and Budwiser 1974). The saliva from semi-engorged females of the tick *Dermacentor variabilis* contains an enzyme acting directly on complement components of normal hosts to produce a substance chemotactic to neutrophils (Berenberg *et al.* 1972). Tatchell and Moorhouse (1970) showed that intense infiltration of neutrophils occurs in *B. microplus* towards the end of feeding, so that a substance chemotactic for neutrophils may be produced later in the life cycle of *B. microplus*.

Hosts of high and low resistance attracted similar numbers of eosinophils into the general area of the attachment site, but there was greater concentration in the immediate vicinity of the attachment site in highly resistant hosts. *In vitro* studies have shown differences between individuals in migration and adherence of neutrophils (Edelson *et al.* 1973), and Talstad (1972) concluded that there was a close correlation between aggregation and phagocytosis. The eosinophils of high-resistance animals might be more responsive to chemotactic factors. In low-resistance animals a number of intact eosinophils remained intravascular. In the high-resistance animals, where this was rarely observed, there may have been preferential attraction of eosinophils. Kay *et al.* (1971) distinguished between vasoactive histamine which allows circulating eosinophils to escape non-specifically from small blood vessels, and the simultaneously liberated ECF-A which selectively attracts eosinophils. That is, there may be a steeper

chemotactic gradient (Wilkinson 1974) in the high-resistance animals. A local concentration of chemotactic factor suggests the retention of antigen which has already been considered in relation to mast cell degranulation.

The considerable variation in eosinophil infiltration between separate lesions, within the same body region of an individual host, could result from two factors. Firstly, although the duration of attachment was reliably measured with radioactive-labelled larvae, there may have been variation in the time taken by the larva to lay down an attachment cone and to penetrate the stratum corneum and other epidermal layers. Secondly, there are some successful larvae on highly resistant hosts and some unsuccessful larvae on hosts of low resistance, so some chance variation in sub-epidermal character such as innervation or vascularity (Schleger and Bean 1971) may locally affect the host response. In unexposed hosts the virtual lack of eosinophil infiltration at the attachment site emphasizes the immunological basis of the reaction. A small amount of degranulation of eosinophils in the subjacent dermal lymphoid tissue of such hosts may reflect unimpaired diffusion of salivary antigens in the absence of an immune response. Primary antigen is taken up by eosinophils in the draining lymph node of mice 1 h after foot pad injection (Roberts 1966).

Two types of degranulation of the eosinophils may occur. Granules discharge into phagosomes (Archer and Hirsch 1963) or the eosinophil lyses and granules are shed (Rothwell and Dineen 1972). During the process of phagocytosis of antigen–antibody complexes, degranulation takes place (Archer and Hirsch 1963; Archer *et al.* 1969). Both phagocytosis by and degranulation of eosinophils have been shown in the presence of antigen–antibody complexes of *Candida albicans* (Ishikawa *et al.* 1972). Both types of degranulation were observed in the present study, with lysis particularly prevalent in the hosts of high resistance. A wide range of enzymes is released from disrupted eosinophil granules. These include ribonuclease, cathepsin, β-glucuronidase and arylsulphatase, whilst peroxidase and acid phosphatase are exposed but remain bound (Archer and Hirsch 1963). These enzymes degrade invading microorganisms (Cline 1972) but they may also cause local tissue damage, as evidenced by the epidermal vesicle in highly resistant hosts, and so cause irritation (Sinclair 1973).

That high levels of resistance of the host were characterized by a greater degree of concentration and subsequent degranulation of eosinophils might be incidental to some other immunological factor which determines the survival of a larva or it might affect survival more directly. Ingestion of the products of degranulation might be toxic to the tick, impair feeding or force migration to a new attachment. Larvae on resistant hosts feed less efficiently and move more often (Kemp *et al.* 1976). Irritation might stimulate grooming which is a factor in the resistance of cattle to *B. microplus* (Snowball 1956; Riek 1962; Bennett 1969). Not only would moving larvae be more susceptible to grooming but an epidermal vesicle under the attachment would facilitate removal.

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