Aust. J. Biol. Sci., 1981, **34**, 27–35

Arteriovenous Anastomoses in the Dermal Vasculature of the Skin of *Bos taurus* Cattle, and their Relationship with Resistance to the Tick, *Boophilus microplus*

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Abstract

Bos taurus cattle with high resistance to the tick *Boophilus microplus*, whether free-grazing or in covered pens, had significantly more arteriovenous anastomoses (AVA) in their skin than did animals of low resistance. These differences in number of AVA associated with resistance level were most marked above the level of the sebaceous gland in the neck region, an area favoured for tick feeding. In this skin layer, the number of AVA in low-resistance animals ($4{\cdot}0 \pm 0{\cdot}4$ per $2{\cdot}1$ mm) was significantly lower than in animals of high resistance ($12{\cdot}3 \pm 2{\cdot}2$ per $2{\cdot}1$ mm) while the mean value for the naive animals ($8{\cdot}2 \pm 1{\cdot}9$ per $2{\cdot}1$ mm) was intermediate. No differences in morphology of AVA were detectable between the three groups using light microscopy.

Introduction

Arteriovenous anastomoses (AVA) are vascular channels connecting the arterial and venous sides of the circulatory system. They are particularly abundant in body extremities so that the prime function attributed to them has been the regulation of body temperature by aiding the dispersal of heat (Grant 1930). In addition, Welbourn (1964) associated them with the regulation of blood pressure and Molyneux (1965) postulated that AVA in the midside region of sheep may be related to the metabolic activity of hair follicles and sweat glands.

AVA were held to be absent from the hairy skin of *Bos taurus* cattle in temperate environments (Goodall 1955; Jenkinson 1965). However, Amakiri (1976) showed that, in a tropical environment, AVA did occur in the hairy skin of both European and Zebu breeds of cattle although they were preponderant in the latter. AVA were also demonstrated in the midside region of Jersey calves from a subtropical environment and the density was found to be greatly enhanced in the pathological condition of hypotrichosis (Schleger *et al.* 1967).

A study of random skin sections from cattle of known resistance to the tick *Boophilus microplus* (Roberts 1968*a*) suggested a relationship between level of resistance and number of AVA. This prompted a more critical assessment of AVA density using thinner sections cut serially.

Materials and Methods

Animals

Two studies involving *B. taurus* cattle from a subtropical environment were carried out. In the first study 12 mature Australian Illawarra Shorthorn (AIS) cattle—11 steers, and one spayed cow—

were individually housed in covered stalls and fed a maintenance ration. These cattle were previously exposed to tick and ranked for resistance level. A daily infestation regime of 1000 larvae to each animal was maintained prior to skin sampling. Additionally six animals, naive with respect to tick, were maintained as a separate group in a tick-free pen and fed similar rations. They were younger than the previously exposed animals but age does not affect the acellular response of naive animals to tick attachment (Schleger *et al.* 1976). The criteria for determining resistance level were as previously reported (Roberts 1968*b*). Animals yielding 0·2% or less of the potential yield of adult females were regarded as being highly resistant, and those yielding 10% or more as having low resistance.

In the second study designed to confirm the results of the first, six free-grazing Hereford × Shorthorn (HS) cows aged 7 years were sampled in February 1976 while under field infestation at Rockhampton, Qld. Three of the animals were of high resistance and three of low resistance. Their resistance level, determined by artificial infestation in January 1976, confirmed their rankings from earlier, more extensive field counts (Sutherst *et al.* 1979).

Experimental Design

In the first study a number of skin characters, in addition to AVA, were demonstrated by histological or histochemical techniques. Because of the urgency of carrying out enzymic reactions the skin of experimental animals, comprising six of high resistance, six of low resistance and six naive with respect to tick exposure, could not be sampled simultaneously. Accordingly, one animal from each of the three groups was sampled on each of six occasions over a period of 1 year. By this means any change in AVA density with time of year could be eliminated from the comparisons. All six HS cows were sampled on the same day in the second study.

In the first study, two skin biopsies were taken with a trephine of 0·5 cm diameter from each of two areas, the neck and the loin. These two areas are favourable and unfavourable, respectively, for tick attachment (Kemp *et al.* 1976). Before sampling, each animal was tranquilized intramuscularly with 0·2–0·8 ml of Rompun per 45 kg bodyweight (Bayer Australia Ltd, Botany, N.S.W.). In the second experiment, the HS cows were sampled in the neck only after the subcutaneous injection of 4·0 ml of 2% (w/v) Xylocain (Astra Chemicals Pty Ltd, North Ryde, N.S.W.).

Histology

The biopsies were fixed in 10% (v/v) alcoholic formalin (Lillie 1965) for at least 48 h, transferred to ascending grades of alcohol, and then to xylene and paraffin, before being embedded in paraffin. Sections 8 μm thick were cut perpendicular to the surface of the skin on a rotary microtome.

Toluidine blue (Mescon *et al.* 1956) and aldehyde fuchsin (Molyneux 1965) were used initially for demonstrating the AVA. The latter stain was found to be the more satisfactory, demonstrating (Fig. 1) the presence of an internal elastic lamina in the artery and its absence in the AVA as described for other species (Welbourn 1964; Abramson 1965; Molyneux 1965). The distribution of mucopolysaccharide throughout the thick wall of the median vessel was characteristic (Molyneux 1977).

Three serial sections were used for the identification of AVA in the skin profile. Each section was examined with a ×25 objective (total magnification ×250), using an eyepiece graticule 1 cm^2 divided into 100 squares each of 1 mm^2. The width of each field, corresponding to the side of the graticule, was equivalent to a section length of 0·42 mm (theoretically 0·40 mm). In the hair-follicle layer, five adjacent fields, equal to 2·1 mm of section length, were examined above the sebaceous gland and five below the sebaceous gland, each field continuing to the full depth of the sweat-gland layer. The locations of apparent AVA and the appropriate field markers, generally individual lobes of the sebaceous gland, were indicated for the middle section on graph paper. The presence of an AVA was either confirmed or discounted after an examination of the two adjacent sections. Below the hair-follicle layer, each section was examined in five bands, each band being a sweep 0·42 mm wide (width of field in hair-follicle layer), perpendicular to the surface of the skin.

Although this technique allowed an accurate assessment of AVA density in the skin profile, it did not permit the expression of this density as number per square centimetre *in vivo*. Such would require the examination of a considerable number of serial sections, either vertical or transverse, to the full depth of the skin for all biopsies. AVA size, which varied considerably in that portion of the hair-follicle layer below the sebaceous-gland level, was not assessed. This was not considered critical as there was little variation in AVA size above the level of the sebaceous gland, the most important layer in the relationship between AVA and tick resistance (see below).

Analysis of Results

The counts of AVA in each of the three skin layers as well as in the total skin profile were examined by analysis of variance to assess the effect of resistance status and body area. The square root transformation was used to equalize the variance. Where the transformation was not entirely satisfactory, due to the greater homogeneity of the animals of low resistance, the Fisher Behrens test for means with unequal variances was used. This was valid as time of year had no significant effect on AVA count in any of the skin layers. Adjustment of counts for the effect of processing on biopsy surface area did not alter the significance of the results.

Results

Morphology

No differences in AVA morphology were observed between animals in the two resistance levels nor between the exposed and naive animals when examined by light microscopy. Examples of AVA found at the level of the sweat gland, as well as below and above the sebaceous gland of a highly resistant, stalled AIS animal, are shown in Figs 2, 3 and 4. In each case the characteristic features of the three main components of the AVA—arteriole, venule and their anastomosis—can be distinguished. As size decreased with proximity to the epidermis, a higher magnification was required to identify the AVA above the sebaceous-gland level.

Table 1. Arteriovenous anastomoses in the hair-follicle layer, above the sebaceous-gland level, between the sebaceous- and sweat-gland levels, and below the hair-follicle layer in skin from the neck and loin areas of naive, and high- and low-resistance Australian Illawarra Shorthorn animals

Value for each layer is the mean ± s.e. of six animals, each animal's value being based on three serial 8-μm sections from each of two biopsies

Resistance level	No. of animals	No. of arteriovenous anastomoses			
		Above sebaceous gland	Sebaceous gland–sweat gland level[A]	Below hair-follicle layer	Total
			Neck		
High	6	12·3±2·2	13·4±2·0	14·2±1·5	39·9±4·4
Low	6	4·0±0·4	8·5±0·8	7·8±1·1	20·3±1·6
Naive	6	8·2±1·9	10·3±0·9	5·6±1·5	24·0±2·2
			Loin		
High	6	14·8±1·6	14·6±1·8	7·4±0·9	36·8±2·6
Low	6	11·3±1·0	10·4±1·3	2·8±1·0	24·5±0·9
Naive	6	11·3±2·6	11·3±0·9	3·1±0·9	25·7±2·9

[A] Large arteriovenous anastomoses often slightly below sweat glands were included in the count.

The sections from one of the free-grazing HS cows contained a considerable number of AVA with a tortuous, striated appearance longitudinally (Fig. 5). The transverse sections of such AVA as illustrated in Fig. 5 (insert), appeared vesicular since the cytoplasm of the epithelial cells was unstained by aldehyde fuchsin. The closely applied, thin-walled collection vein is characteristic.

Density of AVA in Stalled AIS Cattle

The distribution of AVA within and below the hair-follicle layer of the skin as well as between the body areas in the AIS animals of different resistance status is shown in Table 1.

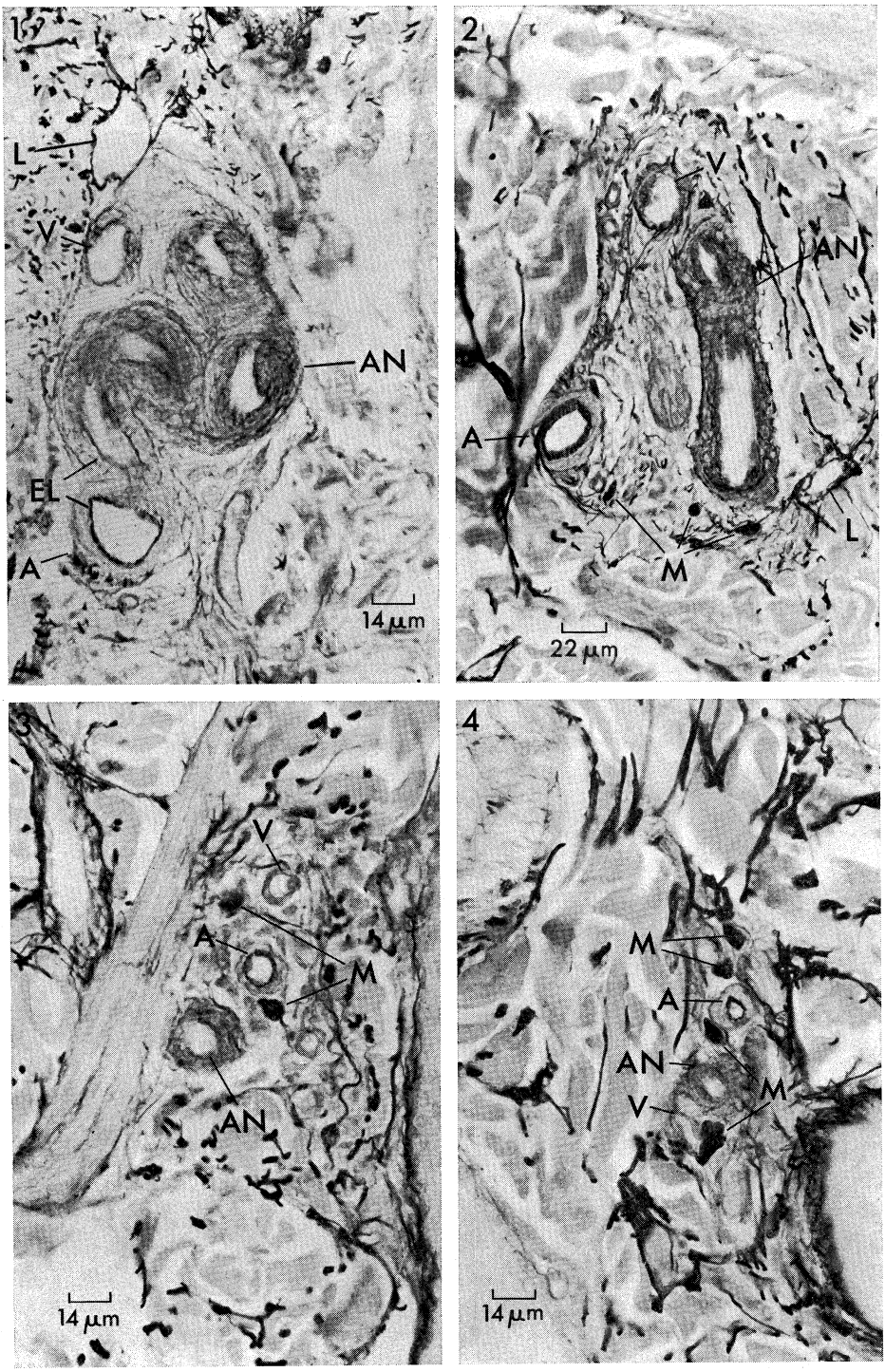

Fig. 1. The thick-walled arteriovenous anastomosis (*AN*), with mucopolysaccharide distributed throughout the muscular wall and with internal elastic lamina terminating at its proximal end, contrasts with the arteriole (*A*) containing a well-defined internal elastic lamina (*EL*), the thin-walled venule (*V*), and the ill-defined wall of the associated lymphatic vessel (*L*). Aldehyde fuchsin.

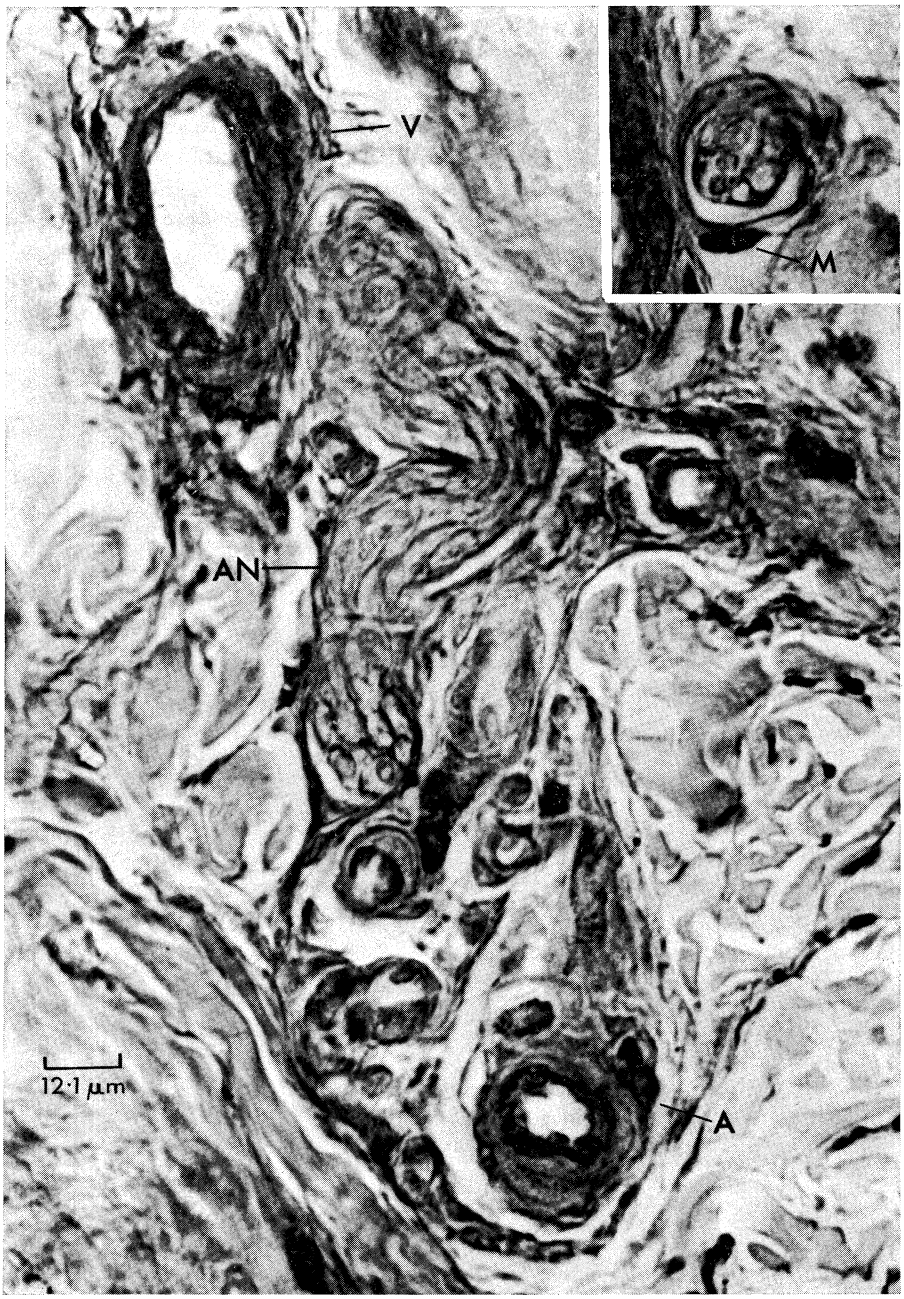

Fig. 5. Anastomosis (*AN*) below the sebaceous-gland level in a highly resistant Hereford × Shorthorn, with a tortuous, striated appearance longitudinally. In transverse section (insert), observed in an adjacent section, the appearance is vesicular with the thin-walled collecting vein closely applied. *A*, artery; *V*, vein; *M*, mast cell. Aldehyde fuchsin.

Figs 2, 3 and 4. Arteriovenous anastomoses at the level of the sweat gland (*2*), sebaceous gland (*3*) and subepidermis (*4*) in an animal of high resistance. In each figure the anastomosis (*AN*), artery (*A*), venule (*V*) and associated mast cells (*M*) can be observed while a lymphatic vessel (*L*) is present in the sweat-gland layer in Fig. 2 as in Fig. 1. Aldehyde fuchsin.

Hair-follicle layer—above the sebaceous-gland level

Table 2 presents the transformed mean, s.e. and standard error of difference (s.e.d.) within resistance levels for counts representing the neck and loin areas for the three groups of animals. The neck area for the animals of low resistance had an AVA density significantly less than that of the loin and less than that of both areas of the animals of high resistance ($P < 0{\cdot}01$) which showed no difference between themselves. The AVA densities of the naive group, which showed an area difference ($P < 0{\cdot}05$), were intermediate in value.

Table 2. Transformed mean, s.e. and s.e.d. for counts of arteriovenous anastomoses above the sebaceous gland in the neck and loin areas of naive and high- and low-resistance Australian Illawarra Shorthorn animals

Resistance level	Counts of arteriovenous anastomoses above sebaceous gland			
	Transformed mean		S.e.	S.e.d. within resistance levels
	Neck	Loin		
High	3·4	3·8	0·25	0·30
Low	2·0	3·3	0·16	0·23
Naive	2·8	3·2	0·34	0·19

Hair-follicle layer—from sebaceous- to sweat-gland level

Differences in counts of AVA in the hair-follicle layer below the sebaceous-gland level (Table 1) were not significant either between resistance levels or between areas.

Below the hair-follicle layer

The estimated mean and s.e.d. between areas and between resistance levels are presented in Table 3 for transformed counts of AVA below the sweat-gland layer in each area and resistance group. The animals of high resistance had significantly greater counts than those of low resistance for both areas. Also, the neck area had a significantly higher AVA count than did the loin for all resistance groups. The naive animals did not differ from the low-resistance group.

Table 3. Transformed estimates for counts of arteriovenous anastomoses below the hair-follicle layer in the neck and loin areas of naive and high- and low-resistance Australian Illawarra Shorthorn animals

S.e.d. between areas was 0·13; s.e.d. between resistance levels was 0·32

Resistance level	Transformed mean of No. of arteriovenous anastomoses below hair-follicle layer	
	Neck	Loin
High	3·7	2·7
Low	2·6	1·6
Naive	2·4	1·4

Whole skin profile

The total counts of AVA for the highly resistant animals ($6{\cdot}1 \pm 0{\cdot}26$ on transformed scale) was significantly greater ($P < 0{\cdot}01$) than that for the animals of low resistance ($4{\cdot}7 \pm 0{\cdot}12$). There was no difference between the neck and the loin

indicating that any deficiency above the sebaceous-gland level in the former was compensated for by a greater number below the sweat-gland level. The naive animals did not differ from those of low resistance.

Hair-follicle layer of the neck in free-grazing HS cows

The mean and s.e.d. of the transformed counts of AVA above the sebaceous-gland level and in the hair-follicle layer as a whole are shown in Table 4 for the neck region in the free-grazing HS cows of high and low resistance. For the counts of AVA to the sebaceous-gland level, as well as the total counts to the sweat glands, the difference between the high- and low-resistance levels was significant ($P < 0{\cdot}05$).

Table 4. Transformed mean for counts of arteriovenous anastomoses in the hair-follicle layer above the sebaceous gland and to the sweat-gland level for the neck region of high- and low-resistance Hereford × Shorthorn cows

Untransformed values are in parentheses

Resistance level	No. of animals	Mean No. of arteriovenous anastomoses	
		Above sebaceous gland	To sweat-gland level
High	3	3·0 (9·5)	4·6 (21·7)
Low	3	1·6 (3·0)	3·0 (9·0)
s.e.d.		0·48	0·45

Discussion

In the neck region of *B. taurus* cattle, an area favoured by the cattle tick, the number of AVA in the hair-follicle layer above the sebaceous-gland level was significantly less in animals of low tick resistance. This relationship between resistance level and AVA count for the neck region of AIS animals confined to stalls was confirmed in free-grazing HS. The counts for the naive group of AIS animals were intermediate in value between those of the two groups of known resistance status. This suggests that the AVA pattern above the sebaceous-gland level might be established before tick exposure, perhaps quite early in life as in human beings (Sherman 1963) or that the number of AVA in highly resistant animals increases with tick challenge.

In the skin profile as a whole the highly resistant AIS animals had a greater density of AVA than the animals of low resistance. This difference, however, was most marked in the neck region above the sebaceous-gland level. This is the location where the cellular response to larval attachment is a good index of an animal's resistance level (Schleger *et al.* 1976).

The inflammatory reaction to tick attachment could cause an increase in AVA numbers. For example, sudden and persistent increases in circulation (Sherman 1963) and injury (Mescon *et al.* 1956) lead to the formation of new AVA. In regions of proliferating capillaries showing congestion and stasis, such as occurs in inflammatory sites (Ryan 1973), new AVA are known to develop. The neck area in animals under tick infestation is such an area. However, a distinction must be made between the intensity of reaction to an individual attachment, greater in highly resistant animals, and the number of successful attachments over all which is obviously greater in

animals of low resistance. The AVA count below the hair-follicle layer was significantly greater in the favoured neck area than in the unfavoured loin for both resistance levels in the AIS and this could reflect an effect of inflammation.

In this study, the distribution of AVA differed from that reported by Amakiri (1976) who described a preponderance of them in the midreticular and midpapillary layers of the dermis. The greatest density of AVA was found in the upper layer of the hair follicle, above the sebaceous-gland level, but they were smaller. On the other hand, the larger and more easily identifiable AVA were found within and immediately below the level of the sweat glands. Large complex AVA were often observed at the dermohypodermal junction as in sheep skin (Molyneux 1977). Simple tubular AVA (Sherman 1963; Ryan 1973) occurred in the upper dermis of one highly resistant animal though classical AVA were found at and below the sebaceous-gland level. Where the upper dermis contained a complex capillary network the identification of glomus-like types of AVA was not easy, a difficulty which has been reported for synovial villi (Lang 1956).

AVA can account for 5–70% of the total circulation (Abramson 1965) and so their high density in the skin of tick-resistant cattle could affect tick survival in several ways. Firstly, dilatation of AVA in the vicinity of the lesion could produce an ischaemic response which is associated with the acquisition of resistance to arthropods (Nelson and Bainborough 1963; Nelson *et al.* 1972). On the other hand, constriction of the AVA could influence the haemodynamic changes (Zweifach 1973) in the inflammatory response to ticks leading to an increased formation of tissue fluid and an increase in lymph flow (Yoffey and Courtice 1970). Thirdly, an emphasis on non-evaporative cooling through the heat-exchange mechanism of the AVA rather than sweating, could produce a relatively low humidity at the skin surface which promotes desiccation of unattached larvae (Roberts 1971; Kemp *et al.* 1976). Although the way in which AVA are associated with tick resistance has not been resolved, they may exert a variety of effects through mechanisms which constitute either physiological adaptation or an immune reaction.

Acknowledgments

The encouragement given by Dr J. A. Roberts in this investigation is gratefully acknowledged and we thank Prof. G. S. Molyneux for helpful discussions.

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Manuscript received 7 April 1979, accepted 19 November 1980