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Comparison of cutaneous hyperemia in cattle elicited by larvae of *Boophilus microplus* and by prostaglandins and other mediators[1]

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Summary. Blood flow has been measured in bovine skin following the injection of tick antigens and a number of pharmacological mediators; including histamine, prostaglandins and slow reacting substance of anaphylaxis. The greatest increase in blood flow (20 times normal) was recorded with tick antigens and with prostaglandin F2. This mediator may therefore influence blood flow during immune reactions to ticks and during the rapid ingestion of blood by the ticks.

An increase in capillary blood flow occurs in cattle *(Bos taurus)* skin at the attachment site of *Boophilus microplus* larvae[2]. On calves without previous tick exposure, an increase was not detected until 24 h post infestation and the peak was not reached until 48 or 72 h post infestation. It was argued that this increase was probably triggered by tick saliva rather than by mechanical damage, since attachments have stabilized[3] and the mouthparts are not found deeply penetrating the dermis at this time[4]. On calves previously exposed to ticks, an increase in cutaneous blood flow was detected as early as 15–30 min after infestation and the flow rate reached a peak at 24 h[2]. The effect of tick feeding on bovine cutaneous blood flow is compared in the present study with the effects of mediators which regulate vascular flow and permeability.

Materials and methods. Cutaneous blood flow was measured using radioactive microspheres (15 µm, diameter, labeled with ^{141}Ce, ^{51}Cr, ^{113}Sn, ^{85}Sr, ^{95}Nb and ^{46}Sc, NEN Co., Boston and 3M Co., St. Paul)[2]. Tick infestation procedures and information on the *B. taurus* cattle used have previously been given[2,5]. All the cattle were 6 months old calves except for animal D (tables 1 and 2) which was 2 years of age. The pharmacological mediators used were prostaglandins (PGE_2 and $PGF_{2\alpha}$), histamine dihydrochloride, histamine free base, 5-hydroxytryptamine creatinine sulphate (5HT), bradykinin triacetate, dopamine, acetylcholine, (all Sigma), adrenalin tartrate (Evans) and slow reacting substance of anaphylaxis ($SRS\text{-}A^{bov}$). The latter was prepared by Dr J.E. O'Hagan, CSIRO, Division of Entomology, from bovine lung[6]. It was assayed on isolated guinea-pig ileum in the presence of atropine and mepyramine, and 1 unit of $SRS\text{-}A^{bov}$ was equivalent to 5 ng histamine base. A serine esterase, designated antigen I, was prepared from *Boophilus microplus* larvae by Dr P. Willadsen, CSIRO, Division of Tropical Animal Science, as already described[7]. It was injected intradermally in 0.1% bovine serum albumin in phosphate buffered saline (PBS pH 7.5) at a concentration of 10 µg/ml. It was known that injection of 1 µg gave a measurable oedematous reaction in most cattle sensitized to the tick.

The mid-flank of the animals was clipped and marked into 4×4 cm areas and each solution was injected into 3 areas. The various pharmacological agents, the tick antigen and a buffered saline control were injected intradermally in 0.1 ml PBS using a 1 ml syringe and 25 gauge needle. The solutions were kept on ice until used. Blood flow was determined at certain times after injection, and as a gauge of increased vascular permeability, weal diameter was measured after 20–30 min. Skin biopsies of injection sites which were taken for blood flow measurements, were sampled after the oedema caused by injection of the mediators had subsided in order to avoid problems with weight changes in the tissues. Some samples were dried to constant weight at 110 °C to check for this source of error[2].

Results and discussion. The first comparison was made between capillary blood flow stimulation by 1000 *B. microplus* larvae, antigen I and relatively large amounts of the various mediators. There was considerable variation be-

tween animals and between sites on the same animal, but PGE_2 was the only mediator which gave a maximum hyperemia (20-fold increase) as large as the response to tick larvae (19-fold increase) in tick-exposed calves (compare tables 1 and 2). Other mediators gave a range of responses from a 8-fold increase by $PGF_2\alpha$, to a reduction to $^1/_{10}$ of the normal blood flow, by adrenalin. As reported in our previous results[2], the cutaneous hyperemia elicited by tick larvae was greater in calves with previous tick experience, particularly during the first 24 h of attachment (table 1).

There was no obvious relationship between a chemical's ability to increase capillary blood flow and to increase vascular permeability (table 2). The extent of oedema was greatest for histamine even with smaller amounts injected. Because histamine was the prime mediator of the bovine oedematous response to *B. microplus* antigen I[8,9], and PGE_2 caused the most potent stimulation of blood flow, they were selected for further study.

The cutaneous blood flow response was much greater with injection of PGE_2 than with histamine (table 3,a). Injection of 0.2 µg histamine had no significant effect but this amount of PGE_2 gave a 5-fold increase in blood flow. It is possible that observations 30 min after injection missed a peak in response to histamine, but observations made at other times after injection still showed a greater response to PGE_2 than to histamine and both reactions had returned almost to control levels by 90 min (table 3,b).

In bovine skin, injection of PGE_2 causes oedema, but in

Table 1. Measurement of bovine skin capillary blood flow at the attachment of approximately 1000 *Boophilus microplus* larvae and at the site of intradermal injection of 1 µg of tick antigen I

Tick dose or antigen injected	Animal No.	Experimental blood flow	Control blood flow	Time after infestation or injection	Weal diameter (mm)	Previous tick exposure
Antigen I (1 µg)	I	58±3	10±3	20 min	22×22	+
	I	57±19	15±6	20 min	20×19	+
	H	47±7	7±1	30 min	28×22	+
	F	31±12	6±1	30 min	19×19	+
	G	6±1	4±0.4	30 min	5×5	−
Larvae 1000	F	131±0.9	7±1	24 h	ND	+
	I	52±10	10±3	24 h	ND	+
	H	46±6	8±0.7	24 h	ND	+
	E	25±4	7±2	24 h	ND	−
	G	5±0.5	3±0.4	24 h	ND	−
	E	64±10	5±2	48 h	ND	−
	G	30±6	4±0.6	48 h	ND	−

Blood flow in ml 100 g^{-1} min^{-1} wet wt of tissue. Weal diameter measured 20–30 min after injection. All figures are the means of 3 replicates.

Table 2. Measurement of bovine skin capillary blood flow at the site of intradermal injection of relatively high concentrations of various mediators and phosphate buffered saline (control)

Mediators and amount injected		Animal No.	Experimental blood flow (±SD)	Control blood flow (min)	Time after injection (min)	Weal diameter (mm)
PGE_2	(25 µg)	D	121±8	6±0.3	30	13
	(20 µg)	P	96±39	7±1	30	21
	(20 µg)	P	67±28	7±2	30	26
	(20 µg)	P	64±18	7±1	34	20
$PGF_2\alpha$	(20 µg)	D	50±4	6±0.3	30	16
	(20 µg)	P	19±16	7±1	20	15
Histamine dihydrochloride	(20 µg)	P	31±3	7±2	30	36
Histamine free base	(10 µg)	K	31±8	15±6	20	28
	(10 µg)	D	12±3	6±0.3	30	29
5 HT	(20 µg)	P	40±5	7±1	16	12
SRS-$A^{bov.*}$	(1000 U)	K	20±2	15±6	20	20
	(1000 U)	D	5±1	5±0.3	30	15
Acetylcholine	(20 µg)	P	19±6	7±1	15	16
Bradykinin	(50 µg)	D	3±0.4	6±0.3	30	16
Dopamine	(20 µg)	P	3±0.6	7±1	17	14
Adrenalin	(20 µg)	P	0.6±0.5	7±1	13	21 Blanched area
Buffered saline control		–	–	–	20	<10

Blood flow in ml 100 g^{-1} min^{-1} wet wt of tissue. Weal diameter measured 20–30 min after injection. 0.1 ml injected at each site. All figures are the means of 3 replicates. *SRS-$A^{bov.}$ 1 unit≡5 ng histamine.

this aspect of inflammation it is less potent than histamine[10]. Our results emphasise the potent effect of 2 PGs in local stimulation of capillary blood flow in cattle skin, and in this, PGE_2 was more effective than $PGF_2\alpha$. Local vascular resistance was shown to vary inversely with blood flow at the injection sites demonstrating a local vascular reaction to the mediators rather than a general effect of the mediators on blood pressure[2]. Both histamine and 5HT injected at high concentrations caused a moderate hyperemia. We are not aware of any reference to the occurrence of 5HT in bovine skin and its effect on blood flow is probably irrelevant to blood flow at the tick attachments.

A local increase in skin capillary blood flow is 1 component of the early host response to tick feeding and to tick antigen injection, but only in calves with previous exposure to the tick[2] (table 1). A comparable level of hyperemia was reached in our experiments only by injection of PGE_2 and it is possible that PGs of host origin have a role in the immediate hypersensitivity reaction associated with tick rejection[11]. Histamine is probably the mediator which causes oedema at the site of tick rejection[9], but PGs are also involved in cutaneous hypersensitivity reactions[12] and they are able to potentiate pain responses[13] and potentiate the exudation induced by other mediators[14].

A complicating factor is that PG is found in the saliva of engorged females of the tick *B. microplus*[15], and PGE_2 has been located in the tick salivary glands, with the highest activity in glands from fully engorged females[16] or from females just prior to final rapid engorgement[17]. It can be estimated that one of these females has approximately 50 ng of PGE_1 equivalents in the salivary glands and hemolymph. It was originally proposed that vasoactive salivary agents might increase the availability of tissue fluids at the start of tick attachment[18] when rate of water loss is high[3,19]. That salivary PGE_2 may be involved at this stage is suggested by the essentially analogous vascular effects produced by the tick in the initial host lesion and by injection of PGE_2[16]. However, in our experiments using the microsphere technique, cutaneous hyperemia was not detected at the time of tick attachment during primary infestation, but was detected 24 h after infestation and was substantial by 48 h (table 1) and 72 h[2] when larvae are feeding rapidly[3].

In seeking an explanation for the increased blood flow so soon after primary infestation, it would not be expected that immunological reactions to the ticks could develop in time to cause the hyperemia observed within 24–72 h of infestation. Antibodies to tick antigens have not been detected in other hosts in less than 7 days after first tick exposure[20–23]. However, there is an accumulation of neutrophils at the larval attachment site on cattle at 48 and 72 h after primary infestation (unpublished information), possibly attracted by chemotactic mediators in the tick feeding lesion[24]. Neutrophils from laboratory animals are thought to contain and release prostaglandins during inflammation[25]. Although there is no information on prostaglandins from cattle neutrophils it is possible that *B. microplus* saliva could trigger the release of mediators from bovine cells.

Our evidence suggests that an increase in local blood flow may be important for the tick during the rapid stage of tick feeding, rather than at attachment. The peak of PGE_2 activity in female salivary glands just prior to rapid engorgement, and the potent effect of PGE_2 on blood flow in cattle skin also suggests that tick salivary PGE_2 is the mediator of increased blood flow in the tick feeding lesion. This would be an interesting adaptation by ticks to promote rapid feeding, but it has still to be shown that PGE_2 is actually secreted from the salivary glands at the time of maximum cutaneous hyperemia, or that increased flow is essential for successful feeding. It is possible that PGs have a different physiological role within the tick[17].

The ability of both salivary glands and reproductive organs of another tick, *Hyalomma anatolicum excavatum* to synthesize PGE_2 and PGF in vitro suggests that prostaglandins may be involved in the feeding and reproduction of ticks[26].

Table 3. Measurement of bovine skin capillary blood flow ml 100 g^{-1} min^{-1} wet wt of tissue; a) 30 min after injection of dilutions of histamine dihydrochloride and PGE_2; b) at different times after injection of 2 µg of histamine dihydrochloride and PGE_2

a) Mediator	Blood flow after different amounts injected*		
	0.2 µg	2.0 µg	20 µg
Histamine	2±2	16±3	29±3
PGE_2	25±6	49±15	78±36

b) Mediator and amount injected	Blood flow at different times after injection*			
	5 min	10 min	30 min	90 min
Histamine 2 µg	8±1	8±2	16±3	4±5
PGE_2 2 µg	-	54±19	61±10	5±6

* Control values subtracted.

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