Our studies provide evidence supporting the supposed close relationship between the Aneuretinae and Dolichoderinae[6-8], as dolichoderine species such as *Iridomyrmex, Tapinoma,* and *Monacis* also produce recruitment pheromones in the sternal gland. Sternal glands are also found in some ponerine, doryline, myrmicine, and formicine species[5], but are different in structure, are frequently located in different anatomical positions, and appear to have evolved convergently. The function of the sternal glands in these groups is largely unknown. It has been suggested that the sternal gland arose de novo in the Dolichoderinae with the specific function of mediating worker communication[3]. The discovery that it is also the source of the trail pheromone in *A. simoni* supports this hypothesis. If the ancestral aneuretines were ecologically similar to *A. simoni* and nested in unstable sites that were frequently disturbed, then the sternal gland may have evolved initially to organize nest emigrations and secondarily have taken on a food recruitment function, as has been postulated in the Dolichoderinae[10].

There is additional evidence linking the Aneuretinae and the Dolichoderinae: the anal glands of dolichoderines are structurally similar to the pygidial glands of *Aneuretus.* Although in both groups they are involved in colony defense, they serve somewhat different purposes. Dolichoderine anal (pygidial) glands secrete substances which alarm colony members and repell intruders[3,9], whereas our experiments with *A. simoni* indicate that the pygidial gland secretion in this species causes aggressive alarm without having a repellent function. But the pygidial gland, like the sternal gland, is not peculiar to the Aneuretinae and the Dolichoderinae, and similar organs have been described in a wide variety of species in all subfamilies including the Nothomyrmeciinae[5,11], a group thought to be ancestral to the Aneuretinae. The discovery of the pygidial gland in *Aneuretus* suggests that these glands are homologous in the Formicidae[5,11]. However, the functions of the pygidial glands are quite different, and in ponerine species they are involved in sexual attraction[12], tandem running[13], and foraging organization[14,15], while in the Myrmicinae they play a role in alarm and defense[16,17].

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Platelet aggregation and stimulation of leucocyte procoagulant activity by rickettsial lipopolysaccharides in rabbits and in man

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Summary. The effects in vitro of 4 purified lipopolysaccharide (LPS) preparations from Rickettsiae on platelets and leucocytes were studied in rabbits and in man. All LPS induced aggregation in rabbit platelet-rich plasma but to differing degrees. This activity was abolished by inactivation of complement. None of the preparations induced aggregation of human platelets. Both rabbit and human leucocytes, when incubated with each of the rickettsial LPS preparations, generated a potent procoagulant activity (tissue factor). These findings add further support to the concept that rickettsial LPS behave as typical LPS from gram-negative bacteria and may be relevant to the understanding of the mechanism(s) responsible for triggering intravascular coagulation in rickettsial diseases.

Recent studies have provided evidence that lipopolysaccharides (LPS) extracted from various members of the genera *Rickettsia* and *Coxiella* are similar to LPS from gram-negative bacteria in chemical composition and in producing some biological effects[2-10]. The capacity of rickettsial LPS to affect the various components of the haemostatic system (contact system, platelets, leucocytes and endothelial cells), a typical feature of LPS from gram-negative bacteria[11-12], so far has not been investigated in spite of the fact that signs of activation of intravascular coagulation (IVC) are seen in association with rickettsial diseases both in man and other animals[13-17].

The present study was undertaken to determine the effects in vitro of various LPS obtained from Rickettsiae on platelets and leucocytes in rabbits and in man.

Materials and methods: Blood collection from normal New Zealand white rabbits and from apparently healthy human subjects, and preparative procedures for platelet-rich plasma (PRP), platelet-poor plasma (PPP), heparinized rabbit PRP, complement inactivation in PPP or serum and for washing of platelets were described previously[18]. Leucocytes (mixed mononuclear cells) were obtained from whole blood by the Ficoll-Hypaque separation technique[19], utilizing 'Lymphoprep' (Nyegaard, Oslo, Norway) as the separation medium. The cells were washed 4 times with Hanks' balanced salt solution (Difco Laboratories, Detroit, Mich., USA) and then resuspended in autologous PPP at the desired concentration. These preparations had minimal granulocyte contamination (< 5%). The ratio of platelets to leucocytes was around 1:1 as determined by light microscopy.

The following LPS preparations were used: LPS from *Rickettsia typhi* (Rt), *Coxiella burnetii* in phase I (CbI), *Coxiella burnetii* in phase II (CbII) and *Rickettsia slovaka*

(Rs), a member of the spotted fever group. These were all highly purified preparations kindly provided by Dr S. Schramek, Institute of Virology, Bratislava, Czechoslovakia. Their isolation and characterization have been described[5-8]. Typical bacterial LPS from *Escherichia coli* 0111:B4 W. and S. minnesota W. were commercial preparations purchased from Difco. All LPS were suspended in sterile isotonic saline by vortex mixing.

Platelet aggregation was studied by aggregometry (aggregometer Elvi 840, Elvi, Milan, Italy) as described previously[18]. The effect of LPS on leucocytes was studied as follows: aliquots of the leucocyte suspension were mixed with each of the LPS preparations or with a similar volume of sterile isotonic saline and incubated at 37 °C. At the intervals indicated the incubates were centrifuged and, after removal of the supernatant, the cells were washed twice, resuspended in Hanks' solution and tested for procoagulant activity. Leucocyte procoagulant activity was evaluated by a 1-stage plasma recalcification time. Citrated human PPP from healthy donors and from individuals congenitally deficient in factor VIII, IX or VII (Dade Division, Pharmaseal, Trieste, Italy) were used as substrates. Rabbit PPP was used for rabbit leucocytes. Clotting time was determined in duplicate in plastic tubes using a mixture of 0.1 ml PPP, 0.1 ml cell suspension and 0.1 ml of 0.025 M $CaCl_2$.

Results. All rickettsial LPS (50 μg/ml final concentration), though to differing extents, induced aggregation in heparinized rabbit PRP (figure 1), but were almost ineffective in citrated PRP suggesting a critical role of divalent cations in these phenomena. Indeed, control experiments revealed that heparin itself, when added at concentrations of 5-10 units/ml to citrated PRP before challenge with LPS, did not influence the aggregation pattern. Aggregation was not seen in PRP stirred with saline for 20 min. None of the LPS induced aggregation in human PRP. When washed rabbit platelets were used, no aggregation occurred. The addition of 20% of rabbit heparinized PPP or fresh heparinized serum to washed platelets restored the aggregating activity of LPS preparations. However, aggregation did not occur when the plasma or serum had been pretreated with hydrazine or adsorbed with zymosan or heated[18] to inactivate complement components.

The preincubation of human leucocytes with LPS (see Materials and methods) resulted in the progressive generation of procoagulant activity as measured by the shortening of plasma recalcification time (figure 2). The table shows the effect of human leucocyte suspensions (5000 leucocytes/μl preincubated with LPS preparations (50 μg/ml final concentration) or isotonic saline for 4 h on plasma recalcification time. LPS-stimulated cells, not control cells, markedly shortened the clotting time of normal, factor VIII- or IX-deficient but not of factor VII-deficient plasma. Rabbit leucocytes, after stimulation with LPS preparations, were also able to markedly shorten the recalcification time of autologous plasma. The range of clotting times (n = 3) was 70-81 sec for Rt, 89-98 sec for RS, 99-115 sec for CbI, 51-64 sec for *E. coli* and 180-320 sec for saline (n = 6).

Discussion. This study demonstrates that LPS preparations from different rickettsial species have various degrees of aggregating activity on rabbit platelets. These effects were observed only in the presence of physiological concentrations of divalent cations (i.e. in heparinized PRP) and of an intact complement system, and therefore were qualitatively similar to those produced by classical LPS from gram-negative bacteria[18,20]. Like many typical LPS[18,20], the rickettsial preparations failed to induce aggregation in human PRP.

In the present study we have also shown that human and rabbit leucocytes generate a potent procoagulant activity when incubated with rickettsial endotoxin preparations. This activity was identified as tissue factor, since it required factor VII for its expression. It was therefore similar to the

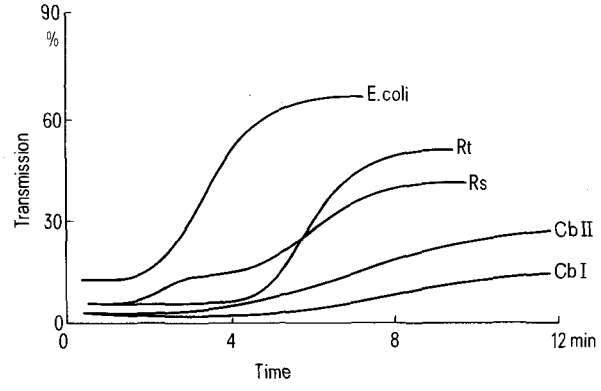

Fig. 1. Aggregation patterns induced by LPS in rabbit heparinized PRP. E. coli *Escherichia coli;* Rt, *Rickettsia typhi;* Rs, Rickettsia slovaka; Cb II, *Coxiella burnetii* phase II; Cb I, *Coxiella burnetii* phase I.

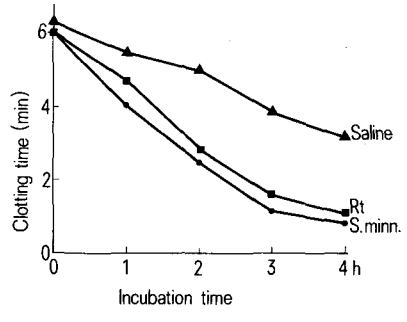

Fig. 2. Effect of human leucocytes preincubated with LPS (50 μg/ml f.c.) or saline on plasma recalcification time. Leucocyte concentration: 5000/μl. Rt, *Rickettsia typhi;* S. minn., *Salmonella minnesota.*

Effect of leucocytes pretreated with LPS or saline on plasma recalcification time

Leucocytes incubated with	No. of experiments	Plasma recalcification time (range in sec)			
		Normal PPP	FVII def.	FIX def.	FVIII def.
Rickettsia typhi	3	63-71	178-224	65-77	60-73
Rickettsia slovaka	3	70-81	208-230	70-86	74-90
Coxiella burnetii, phase II	2	85-96	215-241	83-100	83-106
Coxiella burnetii, phase I	3	90-115	215-235	87-119	89-112
Escherichia coli	4	52-70	174-210	52-73	55-76
Salmonella minnesota	3	49-65	170-203	51-68	47-60
Saline	6	169-300	206-248	175-289	182-244

procoagulant activity generated by human and rabbit leucocytes upon stimulation with classical LPS from gram-negative bacteria[11,12]. Taken altogether these findings add further support to the concept that rickettsial LPS behave as typical LPS from gram-negative bacteria.

Human and experimental rickettsial infections are often associated with clinical and/or laboratory haemostatic disorders consistent with activation of IVC[13-17] but factor(s) responsible for the initiation of IVC have not yet been clearly identified. Emphasis was placed mainly on vasculitis (the fundamental lesion in rickettsial diseases)[16,17] this in view of the variety of potentially injurious mediators produced by the vascular wall (capacity to support platelet adhesion and aggregation, to provide a site for activation of the contact system and to release tissue factor)[12]. Our findings that endotoxins from various rickettsial species stimulate leucocytes to produce a potent trigger of blood coagulation (tissue factor) and the observation that inflammatory cells, particularly mononuclear cells are seen in and around injured vessels[17], suggest an additional mechanism responsible for initiation of local or generalized IVC in rickettsial diseases.

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A putative role for eosinophils in tick rejection

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Summary. In the reaction of *Bos taurus* cattle to infestation by the tick *Boophilus microplus*, mast cell histamine is translocated by the eosinophils to the attachment site. The concentration pattern of this cutaneous mediator for pain appears related to the grooming behaviour of the host.

An association between eosinophilia and both helminth and arthropod infections of mammals has been known for many years. For example, the level of resistance to the tick *Boophilus microplus* in *Bos taurus* cattle is related to the degree of eosinophil concentration and degranulation at the attachment site of the larvae. An effector mechanism in the parasite rejection was thought to be the release of lysosomal enzymes from degranulating eosinophils. These enzymes may cause the tissue damage, and epidermal vesiculation evident in the lesions on highly resistant animals[1]. They could also have a deleterious effect on the tick. For example, in the case of *Schistosoma mansoni*, eosinophils are able to damage the tegument of schistosomulae maintained in vitro[2,3]. The release of hydrolases from degranulating eosinophils, under the influence of complement and antibodies, is believed to be responsible[4]. Another mechanism is possible for tick rejection. As eosinophils contain histamine[5] and also take up histamine from degranulating mast cells[6], it is possible that the infiltration of histamine-containing eosinophils into the tick feeding lesion might play a role in tick rejection. The following experiment shows that eosinophils can concentrate histamine in the tick feeding lesion and that this can be related to the steps in tick rejection.

Materials and methods. A group of 7 animals, 3 of high resistance, 2 of low resistance, and 2 animals, free of tick experience, were used in the experiment. The methods of infestation and determination of resistance status as well as the technique for obtaining skin biopsies of 3 h larval attachment sites have already been described[1]. The biopsies were quenched in liquid propane surrounded by a jacket of liquid nitrogen, freeze-dried for 4 days at $-40\,^{\circ}C$, then embedded in paraffin. Sections of 8 µm were processed for the demonstration of the histamine fluorochrome by the method of Ehinger and Thunberg[7]. Slides were examined in a fluorescence microscope, using the mercury 366 nm line for excitation (UG1 filter) and a Zeiss 41 barrier filter. Attachment sites were identified by the autofluorescence of the larval mouth parts.

Mast cells and eosinophils were identified by their characteristic morphology and granular content[1] which was clearly recognizable in the histamine reaction.

Results and discussion. The histamine reaction shown by the eosinophils in the perivascular cellular infiltrate of control parts of the dermis unaffected by tick attachment sites (figure 1), was weaker than that of eosinophils in the inflammatory area and much weaker than that of mast cells. No concentration of histamine was demonstrated near the tick mouth parts on animals with no prior exposure to ticks. With previously exposed animals[8] histamine was invariably present. At the attachment sites on the 2 animals of low resistance, histamine was confined to the rather sparse dist ibution of intact eosinophils with a small concentration close to the tick (figure 2). At the sites on