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.

1 Reprint requests should be addressed to: Istituto di Microbiologia dell'Università, Policlinico, 70124 Bari, Italy.
2 O.G. Baca and D. Paretsky, Infect. Immun. *9*, 959 (1974).
3 O.G. Baca and D. Paretsky, Infect. Immun. *9*, 939 (1974).
4 M.L. Chan, J. McChesney and D. Paretsky, Infect. Immun. *13*, 1721 (1976).
5 S. Schramek and R. Brezina, Acta virol. *20*, 152 (1976).
6 S. Schramek, R. Brezina and I.V. Taraserich, Acta virol. *20*, 270 (1976).
7 S. Schramek, R. Brezina and J. Kazar, Acta virol. *21*, 439 (1977).
8 S. Schramek, R. Brezina, J. Kazar and T.N. Khavkin, Acta virol. *22*, 509 (1978).
9 A. Paquet, E.D. Rael, D. Klassen, I. Martinez and O.G. Baca, Can. J. Microbiol. *24*, 1616 (1978).
10 D. Fumarola, I. Munno, R. Monno and G. Miragliotta, Acta virol. *24*, 155 (1980).
11 D.C. Morrison and R.J. Ulevitch, Am. J. Path. *93*, 527 (1978).
12 N. Semeraro, in: Hemostasis, Prostaglandins and Renal Diseases, p. 99. Ed. G. Remuzzi, G. Mecca and G. de Gaetano. Raven Press, New York 1980.
13 D. Chernof, New Engl. J. Med. *276*, 1195 (1967).
14 J.R. Graybill, J. Hawiger and R.M. Des Prez, Sth. med. J. *66*, 410 (1973).
15 T. Yamada, P. Harber, G.W. Pettit, D.A. Wing and C.N. Oster, Ann. intern. Med. *88*, 764 (1978).
16 D.H. Walker and W.D. Mattern, Archs intern. Med. *139*, 443 (1979).
17 J.B. Moe, D.F. Mosher, R.H. Kenyon, J.D. White, J.L. Stookey, L.R. Bagley and D.P. Fine, Lab. Invest. *35*, 235 (1976).
18 N. Semeraro, M. Colucci and J. Vermylen, Thromb. Haemost. *41*, 392 (1979).
19 A. Böyum, Scand. J. clin. Lab. Invest. *21*, suppl. 97,77 (1968).
20 N. Semeraro, M. Colucci, D. Fumarola and J. Vermylen, in: Platelets: a multidisciplinary approach, p.293. Ed. G. de Gaetano and S. Garattini. Raven Press, New York 1978.

A putative role for eosinophils in tick rejection

A.V. Schleger, D.T. Lincoln and D.H. Kemp

Divisions of Animal Production and Animal Health, CSIRO, Long Pocket Laboratories, Private Bag No. 3, P.O., Indooroopilly (Queensland, Australia 4068), 10 March 1980

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

animals of high resistance (figure 3) there was a strong reaction for histamine in the central inflammatory area. The distribution of histamine at the 3-h larval lesion was closely associated with the infiltrating eosinophils and the histamine concentration close to the tick was greater than on the animals of low resistance. Eosinophils appear to concentrate histamine in the tick-feeding lesion.

A relevant observation from other studies is that the histamine level in the skin of *Bos taurus* cattle is also greater in animals of greater resistance[9]. In immediate hypersensitivity reactions it has been shown that eosinophils can remove free histamine from the tissue fluids to dampen local inflammation[10]. However in our host - tick interaction they appear to translocate histamine to the tick attachment site.

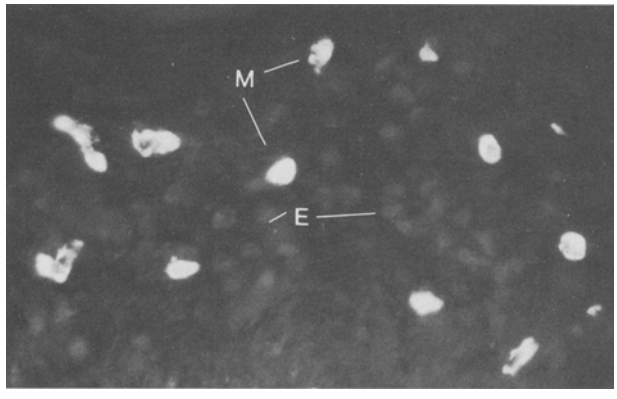
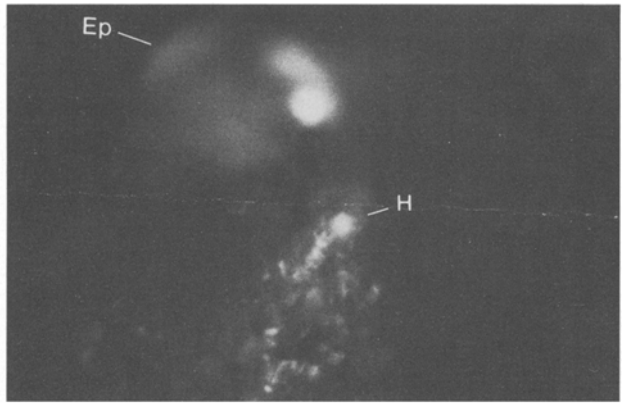
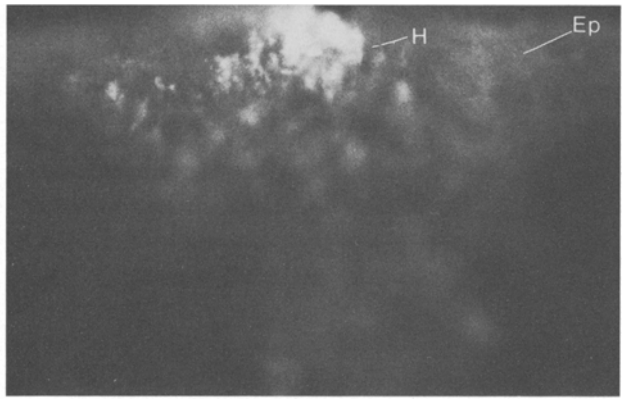


Fig. 1. In the perivascular cellular infiltrate, removed from sites of tick attachment, the faint histamine reaction of the eosinophils (E) contrasts with the relatively intense reaction of the mast cells (M).



2



3

Figs. 2 and 3. The distribution of histamine (H) near the site of a 3-h larval attachment varied between animals. In the low resistance animal (figure 2) there is no concentration in apposition to the epidermis (Ep), most of the histamine reaction being given by intact eosinophils, whereas in the animal of high resistance (figure 3) there is an intense concentration sub-epidermally.

The concentration of histamine in the lesion 2-3 h after tick attachment has an interesting temporal relationship with the behaviour of the tick larvae and of the host. Detachment of larvae from the cattle of high resistance[11] coincides less with the release of histamine through the rapid breakdown of mast cells (0.5 h) than with the optimum time (3-5 h) for eosinophil concentration and degranulation at the target area[1]. This demonstrates a lag in mediator action[6]. The detachment of cattle tick larvae can also be induced by the injection of histamine both in vivo and in vitro[12].

Of greater importance in tick rejection, however, is the grooming response of the host which may be initiated soon after tick attachment, but can be sustained up to 4.5 h after infestation[13]. This can again be related to the time for eosinophil infiltration and degranulation, and may be related to the time required for the concentration of histamine in the lesion. Histamine is regarded as the chemical mediator of cutaneous pain[14]. The importance of histamine in rejection of the tick *Boophilus microplus* has been emphasized by impairment of resistance in some animals following anti-histamine treatment[15]. The presence of an anti-histamine in the salivary glands of another tick, *Rhipicephalus sanguineus,* is of interest[16] in this context.

In the resistance of guinea-pigs to ticks, the infiltration of basophils into the inflammatory area appears to be a critical step in rejection[17-19]. These histamine-rich granulocytes are rarely found in the *B. microplus* - cattle host interaction. Thus, in the absence of a basophil response, an important aspect of the tick rejection mechanism could be the translocation of mast cell histamine to the attachment site by means of eosinophils. On degranulation, these eosinophils appeared to concentrate histamine in proportion to the degree of eosinophilotaxis.

From the suggested role of histamine in tick detachment and host grooming this eosinophil translocation of histamine could be an important step in tick rejection. Other eosinophil constituents, such as lysosomal enzymes, may also be detrimental to tick feeding.

1 A.V. Schleger, D.T. Lincoln, R.V. McKenna, D.H. Kemp and J.A. Roberts, Aust. J. biol. Sci. *29*, 499 (1976).
2 A.E. Butterworth, R.F. Sturrock, V. Houba, A.A.F. Mahmoud, A. Sher and R.H. Rees, Nature *256*, 727 (1975).
3 A.A.F. Mahmoud, K.S. Warren and P.A. Peters, J. exp. Med. *142*, 805 (1975).
4 D.J. McLaren, F.J. Ramalho-Pinto and S.R. Smithers, Parasitology *77*, 313 (1978).
5 L.M. Lichtenstein, in: Cellular and humoral mechanisms in anaphylaxis and allergy, p. 176. Ed. H.Z. Movat. Karger, Basel 1969.
6 T.T. Hubscher, Ann. Allergy *38*, 83 (1977).
7 B. Ehinger and R. Thunberg, Exp. Cell Res. *47*, 116 (1967).
8 J.A. Roberts, J. Parasit. *54*, 657 (1968).
9 P. Willadsen, G.M. Wood and G.A. Riding, Z. ParasitKde. *59*, 87 (1979).
10 E. Goetzl and K.F. Austen, Am. J. trop. Med. Hyg.
11 D.H. Kemp, D. Koudstaal, J.A. Roberts and J.D. Kerr, Parasitology *73*, 123 (1976).
12 D.H. Kemp and A.S. Bourne, Parasitology *80*, 487 (1980).
13 D. Koudstaal, D.H. Kemp and J.D. Kerr, Parasitology *76*, 379 (1978).
14 S.R. Rosenthal, J. invest. Derm. *69*, 98 (1977).
15 R.J. Tatchell and G.F. Bennett, Exp. Parasit. *26*, 369 (1969).
16 W.A. Chinery and E. Ayitey-Smith, Nature *265*, 366 (1977).
17 J.R. Allen, Int. J. Parasit. *3*, 195 (1973).
18 B.G. Bagnall, Ph.D. thesis, University of Sydney, Sydney 1975.
19 P.W. Askenase and M.J. Worms, Fedn Proc. *38*, 1221 (1979).