

the recentrifugation of the pellets in metrizamide gradients (scheme), gives a highly purified granule preparation in the 0.89 M layer. Debris and some pigment, stays at the upper border of this layer.

The overall purification, as determined by the ratio ommochromes: residual nitrogen, is about 49-fold for *Musca* and 24-fold for *Ephestia*, and the recoveries are 10 and 15% respectively (table). For *Ephestia* identical results are obtained, when the calculation is based on pterorhodin. This pteridin pigment is an additional component of the granules<sup>14</sup>. Electronmicroscopic studies reveal, that the preparations are virtually clean with only very little membranous contamination (figure).

The granules of *Musca* represent a type containing only xanthommatin. They show a fibrillar or granular matrix surrounded by a membrane (figure, a). This picture seems to be typical for xanthommatin granules having lost some pigment during fixation<sup>15</sup>. It has been found also for *Calliphora* in situ<sup>4</sup> and after isolation<sup>10</sup>. Under reducing conditions the extinction spectrum of isolated *Musca* granules is identical with that reported for 'red granules' in situ by Strother<sup>16</sup>.

In preparations from *Ephestia*, the eyes of which have a heterogenous ommochrome spectrum consisting of xanthommatin and ommin<sup>17</sup>, most granules (about 99%) are electron dense. Their appearance is very similar to that found in secondary pigment and retinula cells<sup>18</sup>. About 1% of isolated organelles show a fibrous structure typical of depigmented primary pigment cell granulae (figure 1, b). Microspectroscopic measurements on isolated granula preparations under reducing conditions give extinction

spectra identical to those of secondary pigment cells<sup>19</sup>, which contain the mass of ommochromes. Some successful experiments with *Manduca sexta* and *Calliphora erythrocephala* seem to indicate that the method here reported is also suitable for other species.

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## The sternal gland and recruitment communication in the primitive ant *Aneuretus simoni*<sup>1</sup>

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**Summary.** In the primitive ant *Aneuretus simoni*, the only living aneuretine species, recruitment communication is mediated by the secretions of the sternal gland, whereas alarm substances are produced in the pygidial gland. This represents the first demonstration of chemical communication in this species.

Trail communication is nearly ubiquitous in species of the advanced subfamilies of ants, and the source of pheromones releasing trail following behavior varies between groups. In most myrmicine and formicine species trail substances are produced in glands associated with the sting, and the hindgut, respectively<sup>2</sup>, while dolichoderine trail pheromones originate in the ventral organ (Pavan's gland)<sup>3</sup>. The only living representative of the subfamily Aneuretinae, *Aneuretus simoni*, possesses a glandular epithelium in the 7th sternum similar in structure to the dolichoderine ventral organ<sup>4</sup>, now termed the sternal gland<sup>5</sup>. Here we report for the first time on the role of the sternal gland in recruitment communication in *Aneuretus simoni*.

During the course of field studies in Sri Lanka, one of us (AJ) repeatedly observed workers of *A. simoni* traveling along well-defined trails to fallen fruit. Trails were also used during nest emigrations. In the laboratory, scout ants returned to the nest in what appeared to be trail laying posture after discovering a large food source. Inside the nest the scout encountered workers with a motor display which produced arousal. Additional ants soon arrived at the food source.

Trail communication was analyzed by extracting the contents of the hindgut, Dufour's gland, poison gland, pygidial gland, and sternal gland in ethanol, and artificial trails drawn out with a microsyringe were offered to groups of workers. The results (table) show that only sternal gland trails were effective in eliciting trail following behavior. In addition, trails drawn out from nest entrances induced recruitment even when aged 1-2 h prior to testing.

The number of workers following artificial trails 10 cm in length composed of extracts of different glands. Responses were recorded in a 10-min period

Extract tested	Trail					
	I	II	III	IV	V	VI
Sternal gland	38	44	52	37	43	47
Pygidial gland	0	0	1	1	0	0
Dufour's gland	0	0	0	0	0	0
Poison gland	0	0	0	0	0	0
Hindgut	3	1	2	0	0	1

Our studies provide evidence supporting the supposed close relationship between the Aneuretinae and Dolichoderinae<sup>6-8</sup>, 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<sup>5</sup>, 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<sup>3</sup>. 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<sup>10</sup>.

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 repel intruders<sup>3,9</sup>, 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 Nothomyrmecinae<sup>5,11</sup>, 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<sup>5,11</sup>. However, the functions of the pygidial glands are quite different, and in ponerine species they are involved in sexual attraction<sup>12</sup>, tandem running<sup>13</sup>, and foraging organization<sup>14,15</sup>, while in the Myrmicinae they play a role in alarm and defense<sup>16,17</sup>.

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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<sup>2-10</sup>. 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<sup>11-12</sup>, 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<sup>13-17</sup>.

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<sup>18</sup>. Leucocytes (mixed mononuclear cells) were obtained from whole blood by the Ficoll-Hypaque separation technique<sup>19</sup>, 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 slovakia*