Engorgement response of human body lice *Pediculus humanus* (Insecta: Anoplura) to blood fractions and their components

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ABSTRACT. Fed through a synthetic membrane, 80% of females of the human body louse, *Pediculus humanus* de Geer, engorged on whole human blood compared to 30% on platelet poor plasma. Both the plasma albumin and small molecular weight components of the cellular fraction seemed to stimulate engorgement by lice. Known haemo-phagostimulants such as adenosine triphosphate and diphosphoglyceric acid when added to plasma, did not replace the small molecular weight components of the cellular fraction as feeding stimuli. Nymphs discriminated less than adults in selecting blood fractions.

Key words. Body lice, *Pediculus humanus*, artificial feeding, blood fractions, phagostimulation.

Introduction

Blood feeders can be classified into three categories according to their gorging response to blood fractions: those that recognize blood through properties of the plasma and ingest plasma as avidly as whole blood; those that require information limited to the cellular fraction, either red blood cells or platelets, and do not ingest plasma at all unless specific cell components are added to it; and those that are intermediate, feeding on plasma but to a lesser extent than on whole blood, and requiring cellular components for optimal response (Galun, 1986).

The objective of this investigation was to study the gorging response of the body louse *Pediculus humanus* de Geer to blood fractions, and to identify the chemical nature of those fractions used by the louse for recognition of a blood meal.

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Materials and Methods

Lice. Laboratory colonies of human body lice were maintained at 29±2°C and 70–80% r.h. Every other day, lice were placed on the shaved abdomen of a restrained rabbit and allowed to feed to satiety. Nymphs and females used in feeding experiments were starved for 48 h prior to the test. Ten females and twenty nymphs of about the same size, age and nutritional state were placed in each feeding chamber for feeding tests. After 90 min exposure to the diet, engorged lice were counted under a stereomicroscope. Only lice in which the body contents appeared uniformly reddish-brown were recorded as having fed. At the end of each experiment unfed dead lice were discarded. There was a minimum of four replicates of each experiment.

Feeding apparatus. The apparatus consisted of a beaker (4.5 cm diameter, and 4 cm high) containing the experimental solution and a magnetic stirring bar. The feeding chamber (4 cm diameter, 3.5 cm high) was first covered with nylon

netting (1.5 mm mesh) and then with a membrane (see below) held in place with rubber bands; a ring of vaseline kept the lice from escaping. The whole apparatus was placed on a hotplate/stirrer which maintained a membrane temperature of 33±1°C. Ambient temperature was 26±1°C and r.h. 70±10%.

Membrane. 1 g Silicone (Silicone II, General Electric Co., Waterford, N.Y.) was spread on a 10×10 cm parafilm sheet. The silicone was covered with a second sheet of parafilm, distributed evenly by rolling with a cylindrical glass bar. The membranes were dried overnight and cut into nine equal pieces. Silicon parafilm membranes were then stretched in warm air to double their original area and affixed to the feeding chamber.

Solutions. Citrated whole human blood (WB) 0–3 days old, was used in feeding experiments. Citric acid was diluted in sodium phosphate buffer. Red blood cells (RBC) were obtained by centrifuging blood at 300 ***g*** for 12 min, removing the platelet rich plasma (PRP) and the buffy coat, washing the RBC twice in normal saline, centrifuging them at 300 ***g*** each time, and resuspending them in 0.15 M NaCl to the original blood volume. PRP was prepared by centrifuging the blood at 300 ***g*** for 12 min and using the supernatant. Platelet poor plasma (PPP) was prepared by spinning PRP at 1500 ***g*** for 10 min and using the supernatant. Lysed blood (LB) was obtained by thawing frozen whole blood. Plasma filtrate (PF) and lysed blood filtrate (LBF) were prepared by passing the PPP and the LB each through a collodion bag (Sartorius Membranfilter GmbH, Goettingen), cut-off of filter at molecular weight 13,200. Normal saline, 5% bovine albumin in normal saline, Haemaccel® (Behring, Marburg) (3.5% colloidal infusion solution), 6% dextran and 10^{-3} M solutions of ATP, ADP, AMP, DPG (2,3-diphospho-D-glyceric acid) and reduced glutathione (GSH) (all Sigma Co., St Louis, Mo.) in PPP were used for the feeding experiments. The pH of the liquids was adjusted to 7.0 with solid $NaHCO_3$.

Statistics. The results were evaluated by applying the likelihood ratio test to assess difference in goodness of fit of two logistic regression models: (a) the saturated, and (b) the model which assumed no difference between females and nymphs.

Results and Discussion

In experiments on feeding females and nymphs, the order of preference for blood fractions was WB > RBC > PRP > PPP. However, only between WB and PPP was the difference of statistical significance (Table 1). In PPP the important factor seemed to reside in the higher molecular weight fraction, as the PF, which was free of molecules above 13,200 daltons had lost most of its stimulatory activity. The difference between PPP and PF was highly significant ($P<0.001$). In fact, the level of stimulation of PF was the same as that of isotonic saline or water (Table 2). Addition of 5% albumin to the PF or to isotonic saline restored their phagostimulatory effect to that of PPP. Attempts to replace albumin by other plasma extenders such as 6% dextran and Haemaccel®, to determine whether its role is merely that of increasing the viscosity, failed (Table 2). In *Anopheles dirus,* which also requires addition of albumin to saline in order to induce engorgement, albumin could be replaced by other synthetic plasma extenders (Galun *et al.*, 1985). Whether added to saline or to PF, albumin caused approximately the same increase in gorging (11–26% and 10–30%,

TABLE 1. Percentage of body lice engorging on various blood fractions.

	Females		Nymphs	
	%±SD	Total no. tested	%±SD	Total no. tested
Whole blood	79.3±14.8	100	93.3±3.3	77
Red blood cells	61.5±14.4	99	80.2±11.2	84
Platelet rich plasma	52.5±12.6	70	68.1±8.0	100
Platelet poor plasma	30.8±6.9	90	58.9±4.3	78
Plasma filtrate	10.0±0.0	40	40.0±3.5	80

TABLE 2. Percentage engorgement of body lice on solutions of various viscosities and compositions.

	Females		Nymphs	
	%±SD	Total no. tested	%±SD	Total no. tested
Plasma filtrate+5% albumin	30.0±0.0	60	43.8±8.8	80
0.15 M NaCl	11.3±3.8	220	42.5±15.7	377
0.15 M NaCl+5% albumin	25.7±5.5	150	48.1±13.0	56
0.15 M NaCl+6% dextran	10.0±0.0	80	Not tested	
Haemaccel®	6.0±8.8	50	Not tested	
Water	5.0±4.2	40	Not tested	

respectively), indicating that albumin is probably the major phagostimulatory factor in plasma. Other plasma proteins like globulin were not tested to see whether they simulate the effect of albumin.

Addition of LBF to albumin or PPP increased their stimulatory effect significantly (Table 3). This increase can probably be attributed to some small molecules in the cellular fraction of the blood.

Haematophagous insects which recognize blood through cellular components usually use small molecules such as adenine nucleotides as a cue (Friend & Smith, 1977) while ticks use GSH and to a lesser extent ATP and other nucleotides with glucose (Galun & Kindler, 1968). In addition to adenine nucleotides, the reduvid bug, *Rhodnius prolixus*, is also stimulated by DPG (Friend & Smith, 1982). We therefore tested the possibility that ATP, ADP, AMP, DPG or GSH could be recognized by the louse. However, addition of these components to PPP did not increase its stimulatory effect (Table 4).

A significantly ($P<0.01$) higher percentage of

TABLE 3. Percentage engorgement of body lice on lysed blood fractions.

	Females		Nymphs	
	%±SD	Total no. tested	%±SD	Total no. tested
Lysed blood	75.0±0.0	60	71.0±2.2	76
Lysed blood filtrate+PPP	55.0±7.1	60	50.9±5.9	70
Lysed blood filtrate+5% albumin in 0.15 M NaCl	61.3±1.8	60	53.2±2.7	71

TABLE 4. Percentage engorgement of body lice on various haemophagostimulants.

	Females		Nymphs	
	%±SD	Total no. tested	%±SD	Total no. tested
Platelet poor plasma (PPP)	35.0±7.2	40	55.0±17.4	40
PPP+ATP (10^{-3}M)	28.5±2.5	80	60.4±23.2	77
PPP+ADP (10^{-3}M)	35.0±7.1	40	50.0±14.1	40
PPP+AMP (10^{-3}M)	20.0±0.0	40	30.4±12.9	80
PPP+DPG (10^{-3}M)	22.5±3.5	40	53.8±12.9	39
PPP+glutathione (10^{-3}M)	17.5±3.5	40	65.0±7.1	400

nymphs than of adult female lice fed on all blood fractions. They too engorged significantly less on PPP than on cellular fractions but only slightly less on saline than on PPP. Albumin did not appear to play a significant role in their response (Table 2). Addition of known haemophagostimulants to PPP did not increase nymphal gorging on PPP to the level of gorging on blood (Table 4).

The literature on haematophagous arthropods offers several possible explanations for the phenomena reported here. Thus, in ticks, the ratio of whole blood to extravascular fluid ingested has been shown to vary as a function of species, instar (older instars, more erythrocytes) and immune responses of the host (see references in Kemp *et al.*, 1982). For several species of hemimetabolous insects, an increase in the number of chemoreceptors has been reported for each developmental stage (Chapman, 1982). In the present instance, the lesser specificity shown by louse nymphs might possibly be a case in point, though this remains to be demonstrated. Usually nymphs can withstand shorter periods of starvation than adults. The present demonstration that they are less discriminating in their diet may confer on them the advantage of being able to survive on lymph, should this be the only diet available.

Thus lice, like fleas (Galun, 1966), belong to the category of haematophagous insects which show a partial response to plasma and require whole blood for maximal engorgement. The chemical nature of the blood cell components recognized by the louse remains to be determined.

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