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THE AGGREGATION RESPONSE OF HUMAN BODY LOUSE (*PEDICULUS HUMANUS*) (INSECTA: ANOPLURA) TO ITS EXCRETORY PRODUCTS

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Abstract—The human body louse aggregates on filter paper impregnated with an aqueous extract of louse faeces. Chemical analysis of the faeces revealed the presence of haemoglobin, xanthine, hypoxanthine, uric acid and ammonium salts. Of all these compounds, only ammonium salts caused marked aggregation of lice. Excretory products of other insects and ticks also failed to induce aggregation. Total faecal material was more attractive than ammonium, and led to greater aggregation of females than of males. Antennectomized lice reacted neither to faeces extract nor to ammonium carbonate solution.

Key Words: Human body louse, *Pediculus humanus*, aggregation, faeces, ammonium

Résumé—Les poux du corps de l'homme se ressemblent en agrégats sur du papier filtre impregné d'un extrait aqueux de matières fécales de pou. L'analyse chimique des matières fécales révèle la présence d'hémoglobine, xanthine, hypoxanthine, acide urique et de sels d'ammonium. Parmi tous ces éléments, seuls les sels d'ammonium provoquent une forte agrégation des poux. D'autres produits d'excrétion de certains insectes et de tiques ne causent pas d'agrégation. Les matières fécales totales provoquent une agrégation plus forte des femelles que des mâles et leur action est plus marquée que celle des sels d'ammonium. Les poux dont les antennes ont été sectionées ne réagissent ni à l'extrait de matières fécales ni au carbonate d'ammonium en solution.

Mots Clefs: Pou du corps, *Pediculus humanus*, agrégation, matières fécales, ammonium

INTRODUCTION

Excreta of arthopods, haematophagous or otherwise, have been shown to induce conspecific or heterospecific aggregation. Some recently reported examples from different taxonomic groups are: reduviid bugs (Schofield and Patterson, 1977; Neves and Paulini, 1982); soft ticks (Gothe and Kraiss, 1982; Otieno *et al.*, 1985); fruit flies (Prokopy, 1982); beetles (Mitchell *et al.*, 1975); locusts and cockroaches (Shorey, 1976).

Different arthropods apparently produce aggregation stimulating substances in different parts of the body. Some of these substances may be egested in the faeces:

(a) In the German cockroach (*Blatella germanica*) the faeces are contaminated with the pheromone as a result of contact with glandular cells in the rectum, prior to evacuation (Ishii and Kuwahara, 1967).

(b) In the fruit fly (*Rhagoletis pomonella*), on the other hand, the major active component of the pheromone appears to be produced in the midgut, secreted into the gut contents, and released during ovipositor dragging (Prokopy, 1982).

(c) The Malpighian tubules appear to be the site for production of aggregation pheromone which is then excreted into the rectal sac before elimination to the exterior, e.g. guanine in soft ticks (Neitz and Gothe, 1984; Otieno *et al.*, 1985).

For body lice, Wigglesworth (1941) demonstrated that individuals gravitated toward blotting paper soiled with their excreta. The purpose of the present work was to identify chemical components of the faeces of the body louse which induce its aggregation.

MATERIALS AND METHODS

Lice

Colonies of human body lice were maintained at $29 \pm 2°C$ and 70–80% r.h. Every second day, lice were placed on the shaved abdomen of a restrained rabbit and allowed to feed to satiety.

Bioassay

The method of Leahy *et al.* (1973) was used with modification. Whatman No. 4 filter paper circles (9 cm dia) were divided into eight equal sectors and secured in Petri dishes. A 20 μl drop of test solution was placed in one sector, and the same amount of solvent in each of the remaining sectors close to the outside perimeter of the Petri dish, and allowed to dry. Twenty female lice were placed in the centre of the dish and left in darkness at $26 \pm 2°C$, 70% r.h. At the end of 1 hr, the number of lice per sector was recorded. Each test was replicated four times. The following substances were tested: whole human blood and plasma, hemin (hematin chloride), human

and bovine haemoglobin and albumin (5–15% each), ammonium carbonate, ammonium chloride, ammonium sulphate, ammonium nitrate, ammonium phosphate 0.0001–5.0 M, xanthine, hypoxanthine, uric acid, urea, allantoin, allantoic acid, biliverdin and guanine at concentrations from 0.001–0.1 M. For ammonium carbonate and for faeces the response of males and of larvae was compared to the response of females.

Faeces

Louse faeces were collected three times weekly and separated from exuviae and dead lice by sieving. Faeces were added to absolute ethanol, acetone, hexane, ether or 0.02% sodium azide in 0.9% NaCl to constitute 10% w/v. The solutions were stirred overnight at 5°C before being centrifuged for 15 min at 500 ***g***.

Chemical and spectrophotometric analysis of faeces

200 mg of faeces were heated at 50°C in 2 ml of water since some materials were more readily brought into solution at higher temperatures, and stirred magnetically for 30 min. The suspension was filtered through a Buchner funnel, the solid was rinsed with 2 ml water, and the aqueous extract was refrigerated. The aqueous extract of the faeces and the following markers were spotted on 0.1 mm thick silica gel G-60F chromatoplates: hypoxanthine and xanthine were dissolved in water containing a few drops of ammonia; uric acid was dissolved in a dilute solution of sodium hydroxide; urea was dissolved in methanolic sodium hydroxide; and allantoin and allantoic acid were dissolved in warm water. The developing solution was 1% *n*-propanol–1% ammonia (7:3).

Detection of uric acid

TLC of faeces extract was carried out on silica gel = F60 chromatoplates; the developing solvent was *n*-butanol–acetic acid–water (20:7:1). On spraying with bromcresol green, uric acid appears as a blue spot.

The extraction and spectrophotometric determination of haemoglobin

200 mg of dry faeces were mixed with 20 ml of distilled water, stirred thoroughly for 30 min at room temperature, and filtered through a Buchner funnel. The turbid filtrate, reddish in colour, was centrifuged for 10 min at 9000 rpm and measured in a Carry spectrophotometer.

RESULTS

Chemical analysis

Faeces, 100 mg in weight, contained 5.7 mg hypoxanthine and 3.3 xanthine. Both substances appeared as dark spots under u.v. (254 nm) illumination, with R_f values of 0.55 and 0.48 respectively. The aqueous extract of faeces was separated on a 0.25 mm thick preparative thin-layer plate. The bands corresponding to hypoxanthine and xanthine were scraped off the plate and extracted with dilute hydrochloric acid. The u.v. spectrum was recorded on a Contron Uvikon 860 spectrophotometer. The absorption of

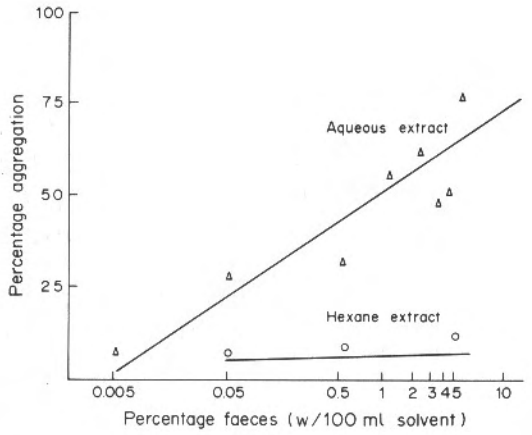

Fig. 1. Aggregation of female lice as a function of concentration of faeces.

hypoxanthine pH 1, was 248 nm and that of xanthine pH 1, was 263 nm.

The uric acid content was 18 mg/100 mg faeces, comprising the major excretory product. The R_f of uric acid was 0.4. No urea, allantoin nor allantoic acid was detected in the extract.

The peak at 405 nm corresponded to haemoglobin. Using a standard solution of haemoglobin (NBC), 200 mg of faeces were found to contain 11.23 mg of haemoglobin. A second peak at 360 nm was not identified.

Chemical analysis revealed that inorganic ammonium salts calculated as NH_4^+ comprise 0.23% of the faeces. The water content of the faeces used for these analyses was 2%. Thus 70% of the faeces are still unaccounted for in chemical terms.

Bioassays

Aggregation was not induced by hexane, ether or acetone extracts of faeces, nor by human blood, plasma, hemin, human or bovine haemoglobin or albumin. Extracts of faeces in absolute ethanol and in 0.9% NaCl caused aggregation, but aqueous extracts gave the best results (Fig. 1). Xanthine, hypoxanthine and uric acid at concentrations of 0.1 to 0.001 M were negative.

Since the tick, *Argas persicus*, showed aggregation in response to guanine, allantoin and allantoic acid (Otieno *et al.*, 1985), and since urea and biliverdin have been found in the faeces of haematophagous insects, these compounds were also tested. None induced aggregation of body lice.

Ammonium, 0.01–0.1 M, led to very effective aggregation (Fig. 2). This range of concentrations corresponds to the range of ammonium salts found in 10% faeces extract (w/v). At higher concentrations, ammonium, particularly in volatile compounds like ammonium carbonate, repelled lice (Fig. 2).

In a two-choice experiment, one sector treated with 10% faeces extract and the opposite sector with 0.05 M ammonium carbonate, almost twice as many lice aggregated on the faeces (47.5%:25.0%).

Faeces were significantly ($P < 0.01$; one-sided Mann–Whitney test); more attractive to females starved for 24 hr than to those recently fed or those

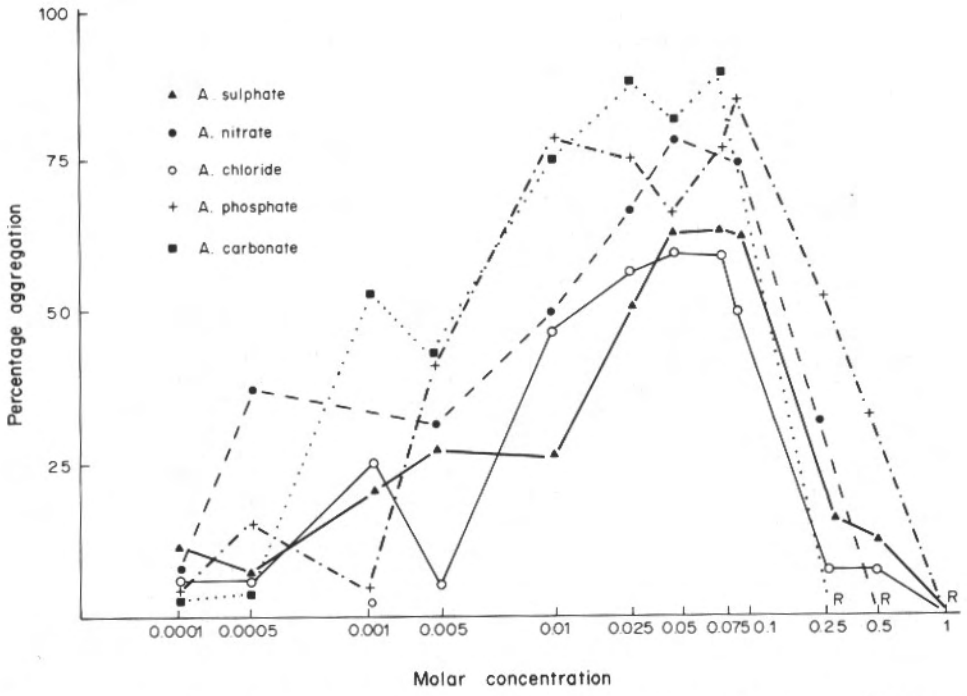

Fig. 2. Aggregation of female lice as a function of concentration of ammonium salts. R = repellent at and above this concentration.

which were fasting for 48 hr. Response to ammonium carbonate was not affected by duration of fasting. Significantly fewer ($P < 0.01$) males and larvae were attracted (Table 1).

Antennectomized lice reacted neither to faeces nor to ammonium carbonate (Table 1). Otherwise they seemed to behave normally as they continued to feed and to oviposit.

DISCUSSION

Female lice show close to 100% aggregation on filter paper treated with a 10% aqueous extract of their excreta. Analysis of this extract showed that haemoglobin, uric acid, hypoxanthine, xanthine and ammonium salts were present. Of these, only the ammonium salts induced aggregation of lice. Ammonium carbonate at a concentration equivalent to that found in 10% aqueous extract produced up to 90% aggregation. In female lice, however, given a choice between faeces extract and ammonium carbonate, more lice aggregated on the faeces extract, indicating that some additional factors are involved. Other metabolites which, tested individually, did not induce aggregation, perhaps act synergistically when they occur together.

The faecal material might, of course, be contaminated with other products of the lice. In the procedures reported here no provision was made to deal with this possibility.

Searching and aggregation behaviours may be differentially affected as a function of degree of repletion in males and females. These behaviours may in turn influence aggregation response.

The possibility that pheromones play a role in the attractivity of louse faeces, cannot be excluded. It is however, apparent that NH_3-releasing components are the major factor in the attractivity and thereby contribute to increased chances of pair formation.

Put to the test, not all pheromones, which by definition are species specific, turn out to be so. Nymphs of *Triatoma infestans* and *Rhodnius prolixus* were found to be attracted to the faeces of either species (Schofield and Patterson, 1977). Ammonium,

Table 1. Response of body lice to ammonium carbonate and to faeces following fasting

Test population	Test solution	Per cent response, hours since last blood meal		
		0	24	48
Females	0.05 M ammonium carbonate	61.2	65.0 (12.5)	60.0
	10% faeces extract	50.0	91.2 (15.0)	53.8
Males	0.05 M ammonium carbonate	50.0	45.0	48.8
	10% faeces extract	55.0	46.3	28.8
Larvae	0.05 M ammonium carbonate	30.0	52.5	30.0
	10% faeces extract	45.0	52.5	30.0

Each test consisted of four replicates containing 20 lice each. Numbers in parentheses indicate the response of antennectomized lice.

associated with the faeces of many arthropod species, haematophagous and otherwise, is proving to be a non-specific attractant, playing a significant role in conspecific and heterospecific aggregations.

Attraction to ammonium is common among many orders of insects and has been used as bait for trapping insects for over 40 years (Dethier, 1947). As pointed out by Dethier, it is easy to understand the attractiveness of ammonium to house flies or other flies which oviposit and feed on manure, but it is more difficult to explain its attractivity for tephritid fruit flies which in mature feed on honeydew and plant sap. Perhaps it may be assumed that attraction to ammonium is a primitive trait evolved from saprophytic ancestors which needed it and is retained through evolution regardless of its behavioural role.

The attraction of lice to the ammonium increases with concentration, reaching an optimum at 0.05 M. Beyond this point attraction decreases with increased concentration, until the nature of the response is completely reversed (repellency). This phenomenon was also observed both in the Mexican boll weevil (*Anthonomus grandis*) and in the house fly (*Musca domestica*) (Dethier, 1947).

It has long been known that chemical insect attractants, especially the sex attractants are detected by means of sense organs located mainly in the antennae (for review see Jacobson, 1972). In body lice it has also been shown that the sense of smell is located in the antennae (Wigglesworth, 1941). Our results with antennectomized females indicate the presence of ammonia receptors on the antennae.

Like insects, ticks also aggregate around sources of ammonia (El-Ziady, 1958). Haggart and Davis (1980) demonstrated electrophysiologically the presence of ammonium sensitive neurons in the dog tick *Rhipicephalus sanguineus*. They believe that low concentrations of ammonium initiate host orientation activity. A similar explanation might apply to the response of the louse. We have demonstrated that both male and female lice aggregate around faeces. One might attribute the resultant meeting of the sexes, usually mediated by pheromones, to the presence of volatile ammonium components of the faeces. Otieno *et al.* (1985) have demonstrated that aggregation of *Argas persicus* and other tick species are a response to guanine, the major nitrogenous metabolite of ticks. This would account for the finding that soft ticks aggregate on filter paper impregnated with excreta of other soft tick species (Leahy *et al.*, 1975). This lack of specificity might have been anticipated in view of the universal secretion of guanine by ticks. Neitz and Gothe (1984) found that guanine did not account for the full aggregation response of *Argas walkerae* and that an additional volatile compound was involved. Perhaps ammonium salts from *A. walkerae* faeces could account for the aggregation behaviour they observed.

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