Although the proposed methods may be effective in reducing maternal cannibalism, they appear to be somewhat cumbersome. Using hypothermia as anaesthetic, we have performed gubernaculotomy in a large series of newborn rats by means of a 4-mm-long transversal incision through the lower abdominal wall[2]. The wound was closed with 6-0 atraumatic silk sutures; 2 were put in the muscle layer and 3 in the skin. After testing various procedures we would like to emphasize the importance of closing the wound very carefully, so that no s.c. tissue is exposed. Moreover, it was necessary to dry away all traces of blood from the skin. Finally, the wound was covered by spraying a thin plastic film (Nobecutan®, Astra). After warming the animals to normal body temperature they were returned to their mothers. There was no special handling of the mothers before or after the operation, and the cages were cleaned within 1 week. When these procedures were used on about 500 newborn rats, less than 5% were lost because of maternal cannibalism.

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The aggregation pheromone of some terrestrial isopod crustaceans

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Summary. Gregariousness has been found in some terrestrial isopod crustaceans. Their faeces were shown to contain the active principle responsible for the initiation and maintenance of aggregation. The active principle was isolated from the mid gut or hind gut and is presumably secreted into the lumen when faecal materials pass and then excreted with the faeces. One of the results of aggregation was shown to be the acceleration of body growth. The active principle was suggested to be an aggregation pheromone.

Gregariousness is common in the animal kingdom. Wilson and Bossert[1] found an active principle which stimulated separate animals to form a group and named it the aggregation pheromone. Since then research on the aggregation pheromone has advanced[2-4]. However, no aggregation pheromone has been reported in invertebrates other than insects, although suggestive results have been found in some species[5-8].

Gregariousness has been found in some terrestrial isopod crustaceans such as the sow bugs, *Porcellionides pruinosus, Porcellio scaber, Alloniscus perconvexus* and *Armadilloniscus tuberculatus,* the pill bugs, *Armadillidium vulgare* and *Tylos granulatus,* and the sea lice, *Ligia exotica* and *Ligidium japonicum.* The present report deals with the behavioural and physiological aspects of aggregation in these isopods.

When a group of these isopods was introduced into a petri dish, after wandering for several min, they aggregated in a certain area of the petri dish and rested. Aggregation was observed in animals kept in the dark or blinded. When the body surface was washed with alcohol, the time necessary for aggregation increased remarkably. Furthermore, when the antennae of these isopods were cut off, no aggregation was observed.

When a piece of filter paper which had been used as a shelter in the stock boxes of these isopods for several days was put into a petri dish in which isopods had been released, the isopods tended to aggregate on this filter paper. This filter paper was contaminated with faeces and presumably odours emitted from the isopods themselves. As the majority of the contamination was faecal, two-choice experiments were adopted for the biological assay. The faeces, about 300 mg in wet weight, were homogenized with acetone in a glass mortar. Then a filter paper was impregnated with the extracted medium. The biological assay was carried out with filter paper impregnated with an acetone extract of the faeces and a clean filter paper. As a result, all the individuals gathered on the filter paper treated with the faecal extract. Thus the active principle responsible for aggregation in these isopods was shown to be contained in the faeces and was presumably communicated by a response to chemical stimuli through their antennae.

A filter paper impregnated with concentrated alcohol washing of the body surface of these isopods was found to elicit a low aggregation response. Although these isopods have many tegmental glands on their cuticular layer, no aggregation activity was found in cuticular extract themselves. Initial aggregation of these isopods appear to be induced by

Bioassay of aggregation pheromone in *Armadillidium vulgare* with Y-maze olfactometer[a]

	Response			
	+	−	Total	χ^2
Control	51	49	100	0.01
Conditioned filter paper[b]	71	29	100	18.91
Faeces[c]	127	73	200	14.05
Faeces in gut*	73	27	100	20.25
Gut**	64	26	90	15.21
Gut (+) and hepatopancreas (−)**	68	12	80	37.81
Fore gut*	39	51	90	1.34
Mid gut*	58	32	90	6.94
Hind gut*	59	31	90	8.10
Ventral part (+) and dorsal part (typhlosole) (−) of mid gut*	41	59	100	2.89

[a] Calculation of χ^2 was made between (+) and (−). Only χ^2 values of 6.64 or greater have significance (p-values of 0.01 or less). [b] This filter paper was contaminated with faeces and odours emitted from the animal themselves. [c] About 1 g in wet weight. * Each sample prepared from 100 individuals. ** Each sample prepared from 20 individuals.

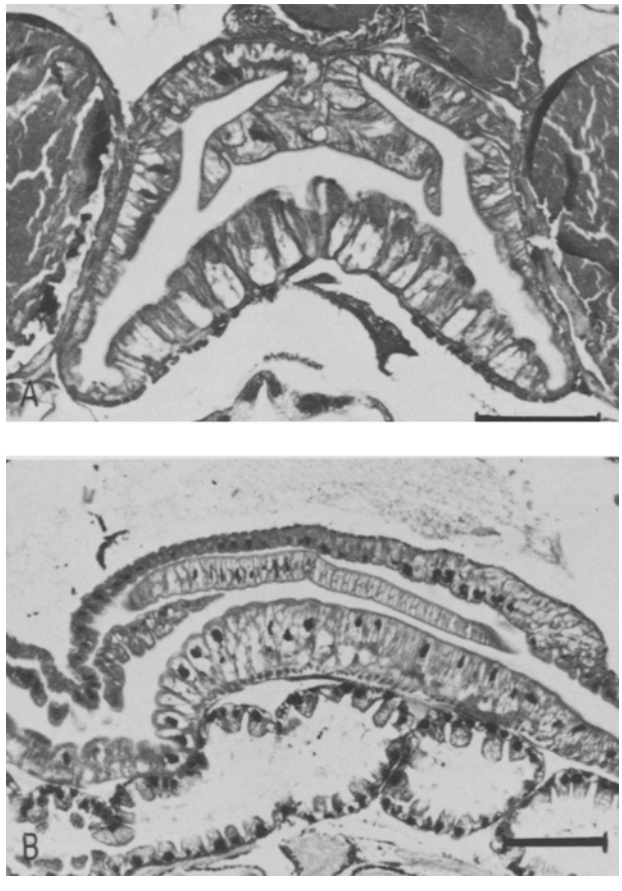
the faecal materials absorbed onto the cuticular layer when faeces are excreted. In addition, the active principle was shown not to be related to the sex of the organism.

In order to determine the secretory site of the active principle, acetone extracts of the samples such as faeces and guts were bioassayed by a Y-maze olfactometer (table). The result indicated that the active principle was mainly localized in the mid- and hind gut. From histological sections, no specialized accessory exocrine glands were found in the mid gut. However, the mid gut consists of large swollen cells in the ventral part, in the ligilate process extending into the lumen and in the typhlosol in the dorsal part (figure). High aggregation activity was found in both ventral and dorsal part extracts. It is suggested that the active principle is produced by these tissues and secreted into the lumen. In *L. exotica,* the active principle is thought to be produced by the ventral swollen tissue near the rectum.

What is the biological meaning of aggregation in these isopods? Body growth under solitary and gregarious conditions was examined. The growth and development of gregarious newly hatched nymphs was more active than in solitary animals.

From these results, the active principle responsible for aggregation is considered to be a pheromone, the aggregation pheromone.

Aggregation pheromones have been demonstrated in social insects such as ants[4] and bees[9], in subsocial insects such as *Lycus*[11], the Coccinellids[12] and many cockroaches[13-18]. Areas of the gut have been implicated as the site of pheromone production in insects: in the aggregation pheromone of Orthoptera[10], the caste differentiation pheromone of Isoptera[19] and the trail pheromone of Hymenoptera[4]. In the German cockroach, *Blattella germanica,* nymphs aggregated in response to a pheromone presented in the faeces and presumably originating from rectal pad cells[13]. In another cockroach, *Blaberus craniifer,* however, the aggregation pheromone was shown to be localized in the mandibular gland[17] and in addition, its chemical nature was demonstrated to be undecane and tetradecane[18]. In the bark beetles, the aggregation pheromone was identified as ipsenol, cisverbenol and ipsdienol in *Ips*[10] and as exobrevicomin, frontalin and ipsdienol in *Dendroctonus*[20]. Furthermore, larvae of the white ant, *Calotermes flavicollis* are known to secrete an attractant substance identified as 3-hexan-1-ol[21]. It is concluded that the active principle responsible for the initiation and maintenance of aggregation in some isopods is secreted from the epithelial tissues of the mid gut or the hind gut into the lumen when faecal materials pass and is secreted with the faeces. This active principle is the first aggregation pheromone to have been demonstrated in invertebrates other than insects.



Histological appearance of the mid gut stained with hematoxylin-eosin in *Armadillidium vulgare. A* Transverse section. *B.* Longitudinal section. Scale bar 200 µm.

* I thank Prof. S. Ishii, Kyoto University, for his guidance and encouragement and to Dr N.W. Runham, University College of North Wales, for his critical reading of the manuscript. A part of this work was supported by Grant from the Itoh Science Foundation.

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