An Aggregation Pheromone of the German Cockroach
*Blattella germanica* L. (Orthoptera: Blattellidae)

I. Site of the Pheromone Production

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Gregariousness has been found in the German cockroach, *Blattella germanica* L. Young nymphs of the German cockroach aggregate when they are resting. When newly hatched nymphs reared in solitary, the growth and development were delayed. The gregariousness is considered to be favourable for the existence of this insect. The fact that the aggregation was observed even in darkness and that the antennae played an important role for the aggregation suggest that chemical stimuli would be responsible. Active principle responsible for the aggregation was found both in the faeces and in ether washing of the body surface. Ether washing of the posterior portion of the abdomen showed higher activity than the other portions. These results indicate that the active principle would be produced either externally or internally in the region of the anus. As the result of histological studies, it was found that the epithelium of the rectum forms six rectal pads consisting of single layer of cells showing glandular nature. Filter papers conditioned with the German cockroaches whose abdominal tips had been amputated did not elicit the response for the aggregation, while those impregnated with methanol extract of the rectums dissected did. It is concluded that the active principle seems to be secreted from the rectal pad cells into the lumen of the rectum when faecal materials are passing, and then excreted with the faeces. The active principle found in the body surface was considered to be of the same origin as found in the faeces, and it adsorbed onto the body surface by its lipid nature. This active principle playing as an attractant for the aggregation seems to be a new kind of pheromone. We offer the term “aggregation pheromone” to this pheromone.

INTRODUCTION

Gregariousness is commonly known in many species of insects. It has also been found in domiciliary cockroaches such as the American cockroach, *Periplaneta americana* L. and the German cockroach, *Blattella germanica* L. (Will, 1920; Gould and Deay, 1938). Gergariousness varies in intensity according to the species and within a species to the age or physiological state of insects.

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Aggregation of the German cockroach nymphs has been believed to depend on an olfactory response to chemical substance produced by the cockroach themselves (PETTIT, 1940; LEDOUX, 1945), and the aggregation favours the growth and development of the nymphs (LANDOWSKI, 1938, WILLIS, RISER and ROTH, 1958). This gregariousness appears to be important in the biology of cockroaches. Although life histories and chemical control methods of cockroaches have been studied by many investigators, physiological aspects on the gregariousness still remain obscure. Roth and WILLIS (1960) comprehensively reviewed on the gregariousness of cockroaches and emphasized that cleverly designed laboratory experiments will be required to elucidate the association of cockroaches in future.

In the course of our study on the gregariousness of the German cockroaches, we found that the aggregation of the nymphs depends largely on a certain chemical substance(s) contained in their faeces and body surface, and this substance serves as an attractant to the cockroaches for the aggregation.

The present experiments deal with the effect of the aggregation on the growth and development of the German cockroach and site of secretion of the active substance responsible for the aggregation.

I. GROWTH PATTERN OF NYMPHS UNDER SOLITARY AND GREGARIOUS CONDITIONS

The growth and development of the German cockroach nymphs were studied under both solitary and gregarious conditions.

Newly hatched nymphs, approximately forty individuals from one ootheca were divided into the following two groups;

Solitary: Twenty newly hatched nymphs were reared individually on dog biscuit (Oriental Yeast Co. Ltd.) as a dietary source and water in small Petri dishes, 2.8 cm in diameter and 1.2 cm in height.

Gregarious: Twenty newly hatched nymphs reared together on the same food and water as for the solitary ones in a Petri dish, 9 cm in diameter and 2 cm in height, being about twenty times larger in the space.

The food was renewed every 2 to 6 days to avoid contamination with moulds. Feeding experiments were carried out at the temperature of 25°C ± 2°C under 14 hr illumination alternating with 10 hr dark in daily rhythm. Each experiment was replicated with two oothcae. The results are shown in Table 1.

As shown in Table 1, the period from egg hatching to maturation of nymphs which were reared under gregarious conditions is faster than that of solitary ones. The gregariousness is more remarkable in younger stages such as the first and second instars than in the later stages. These younger stages elapses faster with simultaneous ecdysis in gregarious than in solitary conditions, although some deviations were observed. There was no significant difference in the body weight of the adults emerged from solitary and gregarious conditions.

II. MODE OF THE AGGREGATION

When a group of the first instar nymphs of the German cockroach was intro-
Table 1. Nymphal Period from Egg Hatching to Adult Emergence of the German Cockroach Reared under Solitary and Gregarious Conditions

<table>
<thead>
<tr>
<th></th>
<th>No. of adults emerged</th>
<th>Mean nymphal period (day)</th>
<th>Mean body weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solitary A</td>
<td>11 (♂ 6 ♀ 5)</td>
<td>65.8 ± 2.4</td>
<td>♀ 49 ♀ 67</td>
</tr>
<tr>
<td>Gregarious</td>
<td>17 (♂ 6 ♀ 11)</td>
<td>58.3 ± 2.0</td>
<td>♀ 52 ♀ 71</td>
</tr>
<tr>
<td>Solitary B</td>
<td>14 (♂ 7 ♀ 7)</td>
<td>65.7 ± 2.6</td>
<td>♀ 51 ♀ 71</td>
</tr>
<tr>
<td>Gregarious</td>
<td>14 (♂ 7 ♀ 7)</td>
<td>55.6 ± 2.2</td>
<td>♀ 50 ♀ 74</td>
</tr>
</tbody>
</table>

Fig. 1. Aggregation of the 1st instar nymphs of the German cockroach, Blattella germanica.

duced into a glass pot, after wandering, the nymphs gathered in a certain area of the pot and rested (Fig. 1).

If a piece of filter paper, which had been used as a shelter in a group of cockroaches for several days, was put into the pot, the nymphs preferred the filter paper conditioned with cockroach themselves for the aggregation. The nymphs crawled in the pot searching for their food and water, thereafter they returned to this aggregation site after feeding or drinking.

The three choice experiment for the aggregation of nymphs was carried out on the conditioned filter paper, 3.5 cm × 7.5 cm, folded in four, and two clean filter papers of the same size as the conditioned one. Some sixty individuals of the first instar nymphs were introduced into a glass pot for the test. As shown in Fig. 2, most of the nymphs aggregated on the conditioned paper.

This aggregation was also observed in the dark. When the antennae of nymphs were cut off, the antennectomized nymphs could not aggregate on the conditioned paper.
Fig. 2. Three choice experiment for the aggregation of the 1st instar nymphs of the German cockroach. A. Approximately 60 individuals were introduced into a glass pot. B. After 19 min. A piece of paper conditioned with cockroaches themselves was placed at upper left. C. After 26 min. D. After 43 min.

These findings indicated that the aggregation was induced by the response to chemical stimuli. The conditioned filter paper was significantly contaminated with their faeces and odour emitted from themselves. When faeces collected from another batch of the german cockroach were affixed with CMC (carboxymethyl cellulose) on a filter paper, the nymphs aggregated on this paper as same as the naturally conditioned paper. Thus, it seems that a certain substance(s) responsible for the aggregation is contained in their own faeces, and stimulates chemical sense through their antennae.

III. METHOD OF BIOLOGICAL ASSAY

In order to localize the site of production of the active principle(s), the following two choice method was adopted for the biological assay. A small piece of filter paper, 3.5 cm x 7.5 cm, folded in four, was conditioned with faeces or extract to be tested. The clean filter paper of the same size as the conditioned one was used as a control. These two filter papers were placed in opposite position in a glass pot, 11 cm in diameter and 7 cm in height. Some twenty to thirty first instar nymphs of the german cockroach were introduced into the center of the glass pot. The biological assay was carried out in the laboratory controlled as same as previously described. The biological assay was usually started in the evening and the nymphs were allowed to prefer any one of the two papers for their aggregating site until next morning without food and water. Each test
was replicated ten times, and results were given in the number of (\(+\)), (\(\pm\)), and (\(-\)): where (\(+\)) indicates most of nymphs aggregate on the conditioned filter paper, (\(\pm\)) indicates about half of the nymphs aggregate on the conditioned filter paper and the remaining half of the nymphs on the control one, or almost none of them aggregate on both conditioned and control filter papers, and (\(-\)) indicates most of them aggregate on the control filter paper.

IV. LOCALIZATION OF ACTIVE PRINCIPLE

It has been known that some secretions were taking place from the body surface of cockroaches. In the preliminary experiment, a filter paper impregnated with concentrated ether washing of the body surface was found to elicit the aggregation response. Therefore, it is necessary to determine whether or not the active principle found in faeces originates from the secretion of the body surface of the german cockroaches.

In order to determine the localization of the active principle, ten to twenty adults of the male german cockroaches were cut into five portions, head, legs, wings, thorax and abdomen. Each portion was washed with ether, and 10 pieces of filter papers impregnated with the ether washings were submitted for the biological assay. Results are shown in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Response(^a)</th>
<th>(-)</th>
<th>(\chi^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>22</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Legs</td>
<td>20</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Wings</td>
<td>28</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Thorax</td>
<td>11</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td>Abdomen</td>
<td>40</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^a\) Result of 5 replications.
\(^b\) Calculation of \(\chi^2\) was made between (\(+\)) and (\(\pm\)) + (\(-\)).

The results indicated that the active principle is mainly localized in the abdomen. A question arose, however, whether the active principle localized in the abdomen, was extracted from internal tissues through the cut opening of the abdomen. Therefore, the abdomen was ligatured between the 1st and 2nd abdominal segments with a fine silk thread just before cutting to prevent the extraction from the interior with ether. The ligatured abdomen had the same effect as the non-ligatured one. It may reasonably be concluded that the active principle was mainly localized on the surface of abdomen, and washable with ether.
To determine the site of production in the abdomen, twenty abdomen of the male adult German cockroaches were transversely cut between the third and sixth abdominal segments, mostly the fifth segment, into two portions, anterior and posterior. These two portions were washed four times each with a small amount of ether respectively. Each ten pieces of filter papers impregnated with anterior and posterior ether washing was submitted for the biological assay. The preference test for the aggregation was performed between two kinds of ether washings of different sources, anterior and posterior portions. The test was replicated two times and the results are given in Table 3.

<table>
<thead>
<tr>
<th>Source of ether washings</th>
<th>No. of filter papers aggregated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior</td>
<td>0</td>
</tr>
<tr>
<td>Posterior</td>
<td>10</td>
</tr>
<tr>
<td>Anterior</td>
<td>2</td>
</tr>
<tr>
<td>Posterior</td>
<td>8</td>
</tr>
</tbody>
</table>

The results indicated that the posterior ether washing is more preferable for the aggregation than the anterior one. As the posterior abdomen seems to be the site of production, twenty posterior portions of the abdomens were transversely cut into further two portions, and each portion was washed with ether and submitted for the biological assay in the same manner as the previous experiment. When the posterior portion of abdomen was cut between 7th and 9th segments, the ether washings of the terminal segments from 7th to the terminal 10th segments was found to be effective for the aggregation as given in Table 4.

<table>
<thead>
<tr>
<th>No. of Replications</th>
<th>+</th>
<th>±</th>
<th>–</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
These experimental evidences supported that the active principle is concentrated in the body surface of the abdominal terminal few segments of the german cockroaches beside their faeces.

Thus, it is important to decide whether the active principle is primarily produced externally in the region of anus and then absorbed onto their faeces, or it is primarily contained in the faeces and then absorbed onto their body surface by its lipid nature.

In order to prevent the reciprocal contamination between the faeces and the body surface, it is necessary to separate faeces from their bodies just after faeces were excreted.

Adults of male german cockroaches were reared on dog biscuit and water in a small cage made by 16 mesh wire-screen, 5 cm × 5 cm × 10 cm, for one week. The wire-screen cage in which the cockroaches were confined was suspended from the top of an insect rearing case 27 cm × 25 cm × 42 cm, and a petri dish was placed on the bottom of the case to collect faeces. The faeces excreted from the caged

german cockroaches were deposited in the petri dish through the wire screen. (Fig. 3). In the first experiment, ten male adults were caged and kept at 25° ± 2°C for one week. One adult excreted about 10 faecal pellets during 24 hr under these conditions.

The faeces collected were extracted with ether and the extract was tested for the aggregation. The caged adults were washed with ether, and the ether washing was also tested.

The second experiment was designed almost the same as the first one except methylene chloride was used for the extraction of faeces.

The extracts of faeces and body washings were found to be attractive for the nymphs. It was confirmed that the active principle was found both in faeces
and in body surfaces.
In addition to the results of the previous experiments in which the active principle was mainly localized on the surfaces of the terminal two or three segments, the present experimental results supported that the active principle was produced in the region of the anus either externally or internally.

V. HISTOLOGICAL STUDIES ON THE SITE OF SECRETION

The abdomen of the adult male German cockroach consists of ten segments as shown in Fig. 4. At the eighth segment, a pair of depressions is located on the abdominal tergite, where secretion occurs at courtship as has been observed by Will (1920) and Roth et al. (1955). These depressions are only found in the male adult but not in the female adult and the nymph. The secretion from the tergal gland lying beneath the epidermis of the depression has no function for the aggregation, because faeces excreted from females and nymphs have the same effect as faeces from males. As secretory organs responsible for the aggregation could not be found externally in the region of the anus of the male German cockroach by microscopic observation, histological studies were undertaken to ascertain the site of the secretion from internal organs.

In the 8th to the terminal 10th segment, there are two main organs, rectum and reproductive organ. The fact that faeces excreted from both sexes of adults and from nymphs are equally effective on the response for the aggregation, suggests that reproductive organs and their accessories are not the sites of the secretion.

Fig. 4. Diagram showing the alimentary canal of the male German cockroach in relation with abdominal segments. A. Dorsal view. B. Ventral view. An: anus, Cr: crop, Cln: colon, Il: ileum, Mal: Malpighian tubules, Rec: rectum, Vent: ventriculus.
Thus, the observation was focused to the hind gut and the anus.

Bouin's solution was mainly used for the fixation of the organs, and paraffin blocks were made in nearly the usual manner. Transverse and longitudinal sections being 5 to 14\(\mu\) in thickness were made, and the sections were stained with haematoxylin-eosin, haematoxylin-phloxin and para-dehydro-fuchsin solutions.

By means of microscopic observations, it was found that epithelium of anterior part of the rectum formed six rectal pads consisted of single layer of cells having large nuclei in the transverse sections as shown in Fig. 5. While in the posterior part of the rectum, there is no such secretory cells as found in the rectal pads (Fig. 6).

The posterior part of the colon, just before the rectum, is a narrow canal, and the epithelial cells are different in their structure from these in the rectal pads, suggesting no function of the secretion (Fig. 7).

The rectal pads were found in both sexes and also in the nymph of the German cockroach, structure of the rectal pad cells of the nymph was as same as that of the adult except small in number and size.

The rectum is located between the eight and tenth abdominal segments as shown in Fig. 4, and is about 1.6 mm in length, 0.7 mm in width in the male adult German cockroach and somewhat larger in the female. Anal glands found in some insects, could not be found in the German cockroach.

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**Fig. 5.** Histological observations on the anterior part of the rectum of the adult German cockroach. A. Transverse section, stained with haematoxylin-eosin. B. Transverse section, the rectum containing faecal matters, stained with haematoxylin-phloxin. C. Longitudinal section, stained with haematoxylin-phloxin. D. Rectal pad, stained with haematoxylin-phloxin. I: intima.
Fig. 6.  A. Transverse section of posterior part of rectum of male adult of the German cockroach. B. Partial enlargement. Stained with haematoxilin-phyloixin.

Fig. 7.  Transverse section of posterior part of colon of male adult of the German cockroach. B. Partial enlargement. Stained with haematoxilin-phyloixin.
VI. BIOLOGICAL ASSAY WITH ABDOMINAL TIP AMPUTATED GERMAN COCKROACHES

If faeces could be excreted without passing through the rectum, nymphs would not respond to these faeces for the aggregation. A fine glass tube was inserted from the anus into the intestine to excrete faeces without passing the rectum, but this attempt was unsuccessful, and faeces could not be excreted.

Transverse cut was made between the eighth and the ninth abdominal segments of the male german cockroaches to remove their abdominal tips where the rectum is located. The cockroaches which had their abdominal tips amputated were released in a glass pot where ten small pieces of filter papers had been placed.

The conditioning of the filter papers with the amputated cockroaches were carried out by supplying dog biscuit and water for 13 to 48 hr. During the conditioning, some of them died, and after 2 days almost all of them died. The conditioned filter papers were submitted to the biological assay for the aggregation by the same method as previously described. The results are given in Table 5.

<table>
<thead>
<tr>
<th>No. of insects for conditioning</th>
<th>Time for conditioning</th>
<th>+</th>
<th>±</th>
<th>–</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>13</td>
<td>0</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>40</td>
<td>18</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>50</td>
<td>24</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>50</td>
<td>48</td>
<td>1</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

As shown in Table 5, the first instar nymphs did not show aggregation response to the conditioned filter papers, but rather showed repellency to them.

The abdominal tips were dissected under a binocular, and the rectums were taken out. The rectums were ground by a small glass rod and extracted with a
small portion of methanol. Filter papers were immersed into the extracts. After
evacuation of the solvent the biological assay was carried out. The results are
given in Table 6.

The results indicated that the nymphs aggregate on the filter papers impreg-
nated with methanol extract of rectums.

DISCUSSION

Gregariousness is commonly known in animals, and its ecological and physiologi-
cal aspects have been studied, and the german cockroach has also been known
to be gregarious. Pettit (1940) studied the gregariousness of the german cock-
roaches and found that nymphs reared in isolation take longer period for their
maturation than those in gregarious condition. He supposed that the altered
metabolism under conditions of crowding and resulting stimulation appears merely
to affect the time required to reach maturity. Ledoux (1945) studied experi-
mentally gregariousness and social interattraction in Blatta orientaris and Blattella
germanica. He concluded that group formation is not the result of chance, but is
a social phenomenon, and that interattraction is mainly olfactory, conditioned by
(1) positive chemotaxis to odours emitted by the cockroaches themselves, (2)
positive hygrotaxis, and (3) thigmotaxis.

Will (1920) observed that nymphs of Blattella germanica remained almost
constantly in groups during the first and second instars, but less so during the
third instar. He believed that the aggregations of young occurred because they
could occupy narrow crevices where the larger insects could not penetrate. At
usual room temperature the older nymphs and adults live completely isolated, but
at certain temperature they gathered together in large, tightly pressed groups.

Landowski (1938) studied on the physiological or psychological effects of greg-
arioousness of Blatta orientalis on the development and growth. He kept nymphs
in groups of 1, 2, 4, 8, and 16 in jars of identical size and shape, and found that
(1) mortality increased with the size of the group and with age, as each animal
occupied more of the available space, (2) life in complete isolation extended the
time required to produce an adult insect, and (3) the mean weight of the adult
insect was, generally, in inverse proportion to the number of nymphs raised
together; isolated insects usually attained the greatest adult weight.

Willis et al. (1958) have also found that Blattella germanica, Blatta orientalis,
and Periplaneta americana complete nymphal development in less time when rear-
ed in groups rather than individually.

The result of the present rearing experiments also confirmed the previous
results that growth and development of the german cockroach was faster when
the nymphs were reared in group. But body weight of newly emerged adults
reared in gregariousness is almost the same as those in solitary. Although
gregariousness had been found in cockroaches, no one studied experimentally the
motivation of aggregation except Ledoux (1945) as cited above. However, he did
not study the emitting site of odours in cockroaches.

Results of the present experiments indicated that the chemical stimuli play an
important role for aggregation, and the chemical substance(s) responsible for the
aggregation was found both in body surface and in faeces. By dissecting the
german cockroaches into head, thorax, abdomen, legs and wings, the active principle(s) was found to be localized mainly in the abdomen.

By further transverse cutting of the abdomen, the active principle was found to be concentrated in the terminal two or three segments of the abdomen. The fact that the active principle was found both in faeces and on body surface seems to suggest the following three possibilities:

1. Two originally different substances from two different sources; one is secreted from the region of anus externally and deposited on the cuticular layer, and the other is secreted from some glands close to the anus internally and deposited on faeces.

2. Originally one substance; which is secreted from the region of anus externally and deposited on faeces.

3. Originally one substance; which is secreted from some glands close to the anus internally and deposited on the cuticular layer of the body.

The hind gut of the german cockroach consists of ileum, colon and rectum and terminates in the anus. The rectum is located between the 8th and the terminal 10th segments. The anterior part of the rectum is narrowed and connected with colon. Based on histological studies, it was found that the posterior part of the rectum is a narrow duct, and the epithelial cells of this part have small nuclei not suggesting any function of the secretion (Fig. 6). In the anterior part of the rectum, six rectal pads space around inside of the rectum sac. Each rectal pad consists of a single layer of cells and is covered with thin layer of the intima. The cells forming the rectal pads are showing columnar shape and elevate on the side toward the lumen, and have large nuclei as shown in Fig. 5. The structure of these cells is showing the secretory nature. The posterior part of the colon is a narrow duct and epithelial cells of this part are quite different in structure from those of the rectal pad as seen in Fig. 7, suggesting no secretory function.

According to SNODGRASS (1935), the function of the rectal pads is not definitely known, and the supposed glandular nature of the organs has never been demonstrated. WIGGLESWORTH (1932) proposed a theory that the organs reabsorb water from the faecal matter in the rectum and thus play an important part in water conservation. This theory was experimentally confirmed in vitro in the american cockroach, _Periplaneta americana_ (WALL, 1967). The present histological observations indicated that the rectum consisted of two parts, anterior and posterior, and the structure of cells surrounding lumen was quite different between two parts as shown in Figs. 5 and 6. The structure of the rectal pad cells in the anterior part does not appear to be particularly adapted for the absorption of water.

Recently BECK and ALEXANDER (1964) studied on the diapause of European corn borer, _Ostrinia nubilalis_, and found a new endocrine system. This hormone named as proctodone is secreted from specialized epithelial cells of the hind gut lying in the 7th and 8th abdominal segments into the haemolymph and appears to affect the brain, resulting in the activation of the brain hormone producing system. No evidence of secretion into the gut lumen was detected.

The sex pheromone of _Ips confusus_ was firstly found in the frass produced by male beetles boring into phloem tissue of the ponderosa pine (WOOD, 1962). This pheromone was supposed to be secreted mainly by hind gut of the male adult,
and was responsible for mass attraction to both sexes (Vité et al., 1963). In mature male beetles, cells having secretory nature were found in the epidermis of the ileum on evidence of histological observations (Pitman and Vité, 1963). Further observations were made on the site of the pheromone production and Malpighian tubules may be concerned with the pheromone synthesis (Pitman et al. 1965), but this has yet to be established firmly. They also found that faecal pellets excreted from male beetles elicit a clear response for the attraction and that the pheromone is becoming concentrated in the rectal region of the alimental canal. However, the actual localization of the pheromone site still remains obscure (Wood et al., 1966).

Blum and Portocarrero (1964) found that the trail pheromone of the neotropical army ant, Eciton hamatum, is secreted by the hind gut. However, it is still not known whether the pheromone is produced by special cells in the hind gut or is a product of digestion formed more anteriorly in the digestive tract.

In the present experiments, the fact that ether extract of the food did not elicit the response for the aggregation indicates the food material has no activity for the aggregation. The digestion products still pose a possibility for the aggregation. However, filter papers conditioned with the German cockroaches whose abdominal tips had been cut off at the part between the 8th and 9th abdominal segments did not show any activity to elicit the aggregation response as given in Table 5. Moreover, faeces excreted from the German cockroaches which have been reared on a different food elicit response. These experimental results seem to deny the possibility mentioned above.

This active principle responsible for the aggregation of the German cockroach seems to be a new pheromone, and we offer to name this pheromone as an "aggregation pheromone".

It may be concluded that the aggregation pheromone is produced in the rectal pad cells and emitted into the lumen when faecal materials are passing through the rectum. The active principle found in the surface of abdominal terminal segments is considered to be of the same origin, and is possibly adsorbed onto the cuticular layer in the region of the anus by contamination with faeces when these are excreting. After excretion of the faeces, the pheromone contained in faeces seems to have some volatility, it would be absorbed on the surface of insect body by its lipid nature.

The aggregation of the German cockroaches was clearly initiated by olfactory response to this pheromone serving as an attractant, but the maintenance of the aggregation may be concerned with other factors.

Isolation of the active principle is now in progress, results will be published in the proceeding papers.

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REFERENCES


