b formed respectively during incubation, differ significantly in their mobility. The cytoplasmic enzyme carries more negative charge and migrates farther towards the anode during electrophoresis. This is consistent with the earlier findings 1,2 that the cytoplasmic enzyme is more strongly adsorbed on DEAE cellulose than the mitochondrial enzyme. The weak formazan band b', which appeared in the cytoplasmic fraction and which corresponded in electrophoretic position with band b in the mitochondrial fraction, may be interpreted as the enzyme activity of mitochondrial origin. There is evidence that the mitochondrial enzyme is easily leached into the soluble phase during isolation<sup>1</sup>. An additional band of enzyme activity c, however, was also detected near the origin in the mitochondrial fraction; this activity was shown to be substrate-dependent. It would thus appear that the mitochondrial fraction of barley contains 2 malate dehydrogenase activities. Nevertheless, the present investigation clearly demonstrates that the cytoplasmic and mitochondrial enzymes are electrophoretically distinct, as well as supplementing the earlier observations<sup>1,2</sup> that the 2 enzymes differ in chromatographic behaviour. The findings also suggest that the mitochondrial malate dehydrogenase in barley in itself is electrophoretically heterogeneous, although the possibility exists that this additional activity is formed as a result of extraction and separation. Similar electrophoretic pattern of the malate dehydrogenase on starch gel has also been recently reported in maize 6,7.

Zusammenfassung. Malatdehydrogenase aus der Mitochondrienfraktion von Gerstenkeimlingen verhält sich elektrophoretisch verschieden von der Malatdehydrogenase aus der Cytoplasmafraktion.

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## Aggregation of German Cockroach (Blattella germanica) Nymphs

Young nymphs of the German cockroach Blattella germanica L. are known to be gregarious and their aggregation is believed to depend largely on an olfactory response to chemical substances produced by the cockroaches themselves 1,2. When young nymphs are introduced into a clean glass container, they aggregate in one area. They leave this area to search for food and water but return to it again after feeding or drinking. This gregarious behaviour is shown best by first and second instar nymphs and appears to be important in the biology of the species. As previously reported by WILLIS et al.<sup>3</sup> the growth rate of young nymphs reared individually is slower than that of nymphs reared in groups. We also confirmed that the growth rate is increased when the nymphs are allowed to form aggregations. The gregarious behaviour of cockroaches has been comprehensively reviewed by Roth and WILLIS<sup>4</sup>. The present communication describes experiments designed to determine the site of secretion of the active material(s) responsible for the aggregation.

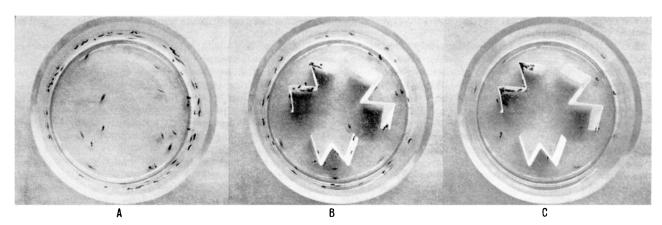
Active material was shown to be present in the faeces. A group of German cockroaches was allowed to shelter in a small piece of filter paper  $(3.5 \times 7.3 \text{ cm})$  folded in 4, during which time the paper became conditioned by contamination with faeces. Some 30-60 first instar nymphs were introduced into a clean glass container (11 cm in diameter and 6.5 cm in height) containing the conditioned filter paper and 2 clean pieces of the same size as the conditioned piece. The aggregation behaviour of the nymphs was observed. As shown in the Figure, most of the nymphs had aggregated in contact with the conditioned paper after about 40 min. Similar results were obtained with filter paper to which faecal pellets had been affixed with carboxymethyl cellulose. Aggregations of B. germanica also occurred to some extent in response to the

faeces of the other species of cockroaches such as *Periplaneta americana* L. and *P. fuliginosa* (Serville) but not to those of the larvae of the silkworm, *Bombyx mori* L.

Aggregations also occurred in response to ether washings of the body surface. When surface washings of head, wings, legs, thorax and abdomen were tested for activity, those of the abdomen were found to have the highest activity to elicit the aggregation response. The possibility that the active material may have been extracted from the cut anterior end of the abdomen was excluded by the finding of activity in washings from abdomens which had had the anterior end sealed by ligation before being excised from the thorax. When the abdomen was divided into 2 parts by a transverse cut made between the sixth and eighth segments, the ether washing of the posterior portion was found to be more active than the anterior one.

The colon, rectum and anal region were sectioned and stained with hematoxylin-eosin, paraldehyde-fuchsin and chrome alum hematoxylin-phloxin in an attempt to identify the site of production of the pheromone. The colon and anal region seem to be devoid of secretory cells but the rectum possess 6 thick pads made up a single layer of cells with large nuclei and having an appearance

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Three choice test for aggregation of the first nymphs of the German cockroach. (A) A group of the nymphs, approximately 60 individuals, was introduced into a glass pot. (B) After 19 min. A piece of filter paper conditioned by contamination with cockroach faeces was located at upper left. (C) After 43 min.

suggestive of secretory activity as previously supporsed by Snodgrass<sup>5</sup>.

Small pieces of filter paper conditioned by contact with male cockroaches which had been deprived of the eighth, ninth and tenth abdominal segments did not elicit the aggregation response. Nymphs did aggregate, however, in response to filter paper impregnated with a methanol extract of the rectum and posterior part of the colon.

The results indicate that material having the activity of an aggregation pheromone is possibly produced in the rectum and that it is applied to the faeces as they emerge. The activity on the surface of the abdomen is presumably from the same source, the spread of the active substances over the abdominal surface being facilitated by the fluid nature of the cuticular wax.

Cockroaches which had had their antennae amputated did not aggregate. This finding, together with the observation that aggregation occurs on conditioned filter paper even in darkness, confirms the supposition that the olfactory sense plays an important role in the aggregation behaviour.

It has recently been known that pheromones serve as attractants responsible for aggregations of Lycus loripes (Chevrolat)<sup>6</sup>, Ips confusus Lec.<sup>7</sup>, and Calotermes flaviollis (Fabr.)<sup>8</sup>. The meaning of the aggregation is, however, different in the insect species studied.

The aggregation pheromone found in the German cockroach also seems to serve as attractant for the aggregation. It is evident that this pheromone is contained in faeces excreted from the cockroach themselves without regard

to sex and nymphal stages, and that the gregarious behaviour of nymphys favours their growth and development. -A more extensive paper will be published elsewhere.

Zusammenfassung. Die Larven der Schabe, Blattella germanica L., leben in Verbänden, wodurch ihre Wachstumsgeschwindigkeit beschleunigt wird. Der Herdeninstinkt funktioniert auch im Dunkel, nicht aber nach Abschneiden der Fühler. Eine chemische Erregungssubstanz, die für Zusammensitzen verantwortlich ist, wurde im Kot gefunden. Diese wird offenbar im Rectum produziert und im Kot ausgeschieden. Die Substanz wird als eine Art von «Pheromon» angesehen, und es wird vorgeschlagen, da sie für das Zusammensitzen der Schaben verantwortlich ist, sie als «Aggregationspheromon» zu bezeichnen.

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## Variations of Nucleic Acid Content in the Salivary Glands of *Drosophila hydei* during Late Larval Development

The DNA and RNA content of the salivary glands of *Drosophila* has been previously investigated by Patterson and Dackerman<sup>1</sup>, and Chen et al.<sup>2</sup>, by biochemical methods, as well as, for DNA, by histophotometry <sup>3-5</sup>. All these works were carried out with the purpose of detecting the absolute values of the nucleic acid content in the mature gland, or in order to make a comparison between cells of different genotypes.

The only information so far available on the variations of the nucleic acids content in the salivary glands during their differentiation, comes from a recent research by RODMAN<sup>6</sup> who has been able to follow the changes in the amount of DNA with the aid of histophotometry.

The investigations reported in the present paper concern the determination by biochemical methods of the nucleic acid content in the salivary gland during the period in which the late differentiation of the gland takes place in connection with the increased nuclear politeny<sup>5</sup>. For this purpose, from synchronized<sup>7</sup> cultures of *Drosophila hydei* St. (wild stock), we collected a consistent number of larvae every 24 h, from the late second to the late third instar. Biochemical determinations of DNA and