

In similar experiments in which over 30 *C. cautella* were examined, temperature gradients ranging from about 17–32°C were used without food present in the channel apparatus. Adults (females) were 1–3 days old and had been bred at 25°C and 65–70% R.H. Observations were taken at 5 min intervals for about 6 days. Representative data is given in Figure 1C where it can be seen that the female adult spent alternate periods in the cool region of the gradient interspersed with periods, of a much shorter duration, in a warmer region. Oviposition occurred in the warm region of the gradient where the adult was also relatively more active.

Although the experiments reported above can only be considered as exploratory, it is interesting to note that GRAHAM⁵ inferred from his population gradient studies that some form of cyclic behaviour was present in *T. castaneum*. Cyclic behaviour has also been observed in *Pseudophonus pubescens* for example, where daily and seasonal periodicities were present⁶. There appears to be no evidence in the literature suggesting cyclic behaviour of *C. cautella* on a temperature gradient. Further work is planned to investigate the nature of this cyclic behaviour and it is also proposed to examine other stages in the life cycle, such as the larval stage, to determine if a similar phenomenon exists⁷.

Résumé. Des observations faites en laboratoire sur le comportement d'individus adultes de *Tribolium castaneum* et *Cadra cautella* dans des zones de températures différentes montrent que ces insectes ne se trouvent pas indéfiniment dans la même zone, mais ont la tendance de séjourner alternativement dans une zone chaude et une zone froide. Ces déplacements semblent avoir un caractère rythmique.

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⁷ This work forms part of the research programme of the Department of Natural History and was carried out in collaboration with the Tropical Stored Products Centre, Ministry of Overseas Development, Slough.

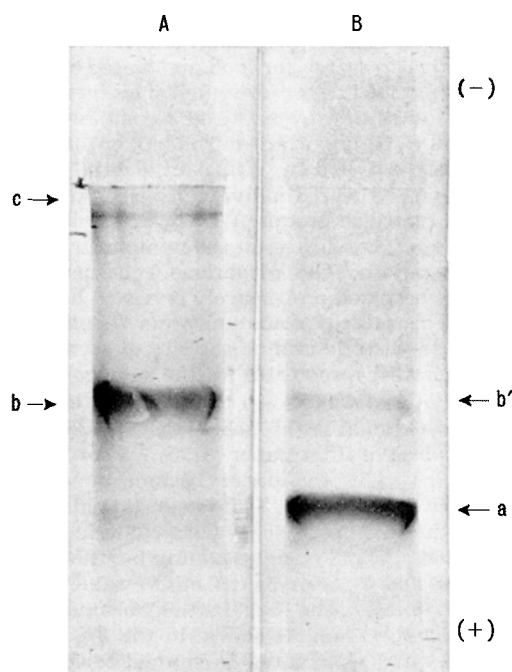
⁸ On transfer from Tropical Stored Products Centre, Ministry of Overseas Development, Slough, Bucks.

Electrophoretic Mobilities of Malate Dehydrogenases from Barley Seedlings

We have recently reported¹ the occurrence in young barley seedlings of 2 malate dehydrogenases (L-malate: NAD oxidoreductase, 1.1.1.37), one being associated with the cytoplasmic fraction and the other a particulate, presumably mitochondrial, fraction of the cell. The 2 enzymes have been shown to differ in chromatographic behaviour on diethylaminoethyl (DEAE) cellulose and in their substrate affinities¹. Similar correlation between intracellular origin and chromatographic behaviour of malate dehydrogenases has also been reported for other plant tissues². In the present study, the 2 enzymes have been compared as regards their electrophoretic mobility with a view to further characterizing their physical properties. Electrophoresis of the 2 subcellular fractions of barley seedlings on polyacrylamide gel, followed by detection of the enzyme activity by tetrazolium staining, revealed that the 2 malate dehydrogenases are electrophoretically heterogeneous.

The cytoplasmic and mitochondrial fractions of young barley (*Hordeum sativum*) seedlings were prepared as previously described¹. Electrophoresis³ of the fractions on polyacrylamide gel was carried out at 4°C. Aliquots of samples containing 20–200 µg protein, determined by the standard KJELDAHL procedure⁴ after precipitation with trichloroacetic acid, were each layered on top of the spacer gel prior to electrophoresis. Good resolutions of the enzymes were obtained by employing a current of 2 mA per column for 15 min and then 5 mA for 30 min in Tris-glycine buffer, pH 8.6. Malate dehydrogenase activity was detected by the tetrazolium technique⁵. The reaction mixture contained 1 vol 1.0 M sodium malate; 1 vol 2 mg/ml phenazine methosulphate; 1 vol 5 mg/ml nitro blue tetrazolium; 1 vol 0.1 M sodium cyanide; 4 vol 0.75 mg/ml nicotinamide adenine dinucleotide; and 2 vol 0.1 M phosphate buffer, pH 6.0.

It is clear from the Figure that the cytoplasmic and mitochondrial malate dehydrogenases, the activity of which being detected by the purple formazan bands a and



Electrophoresis patterns of malate dehydrogenases from barley seedlings. (A) Mitochondrial fraction. (B) Cytoplasmic fraction. These gels were simultaneously subjected to electrophoresis and incubated in the reaction mixture for the same period of time.

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 isolation¹. 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^{1,2} 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.³ 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 WILLIS⁴. 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 × 7.3 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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