Table 1. Time expended on components of predatory behavior of the ladybeetle, *Coccinella septempunctata*

	Component		
	Feeding bout ^a	Grooming	Stationary
Mean duration of a component (min)	3.2±3.3 ^b	4.0±3.3	9.1±10.4
% Expended on each component	25.2±13.5	33.8±16.5	41.0±18.1
No. of components during observation	4.7± 1.7	5.2± 2.1	2.9± 1.2

^a Feeding bout includes searching and capture, both of which were of short duration.

^b Values are means±S.D.

prey when there was none.

It is suggested that the most frequent sequence of the predatory behavior of the ladybeetle is: searching—capture—consumption—searching and that grooming often appears after consumption (Fig. 1.). After grooming, the beetle will again search for a prey. However, if satiated it will simply become stationary.

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Preliminary Notes on Glycosidases in Two Cell Lines Derived from the Ovary of the Cabbage Armyworm, *Mamestra brassicae* (Lepidoptera: Noctuidae)¹

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Characterization of the enzymes in insect cell lines provides useful informations not only for clarifying the function of the enzymes, but also for distinguishing cell lines from each other. However, only a few attempts have been made to study the enzymes of cultured insect cells. The present

paper reports the results of preliminary experiments on several glycosidases found in the cells and the culture media after the two cell lines were cultured.

The cell lines used in this experiment were NIAS-MB-19 and NIAS-MB-32, both derived from pupal ovaries of the cabbage armyworm, *Mamestra brassicae* (MITSUHASHI, 1977). In brief, each cell line was cultured in 30 ml of MM medium (MITSUHASHI and MARAMOROSCH, 1964) containing 3% fetal bovine serum (FBS) at 25°C. After a week of cultivation, the cells were harvested, spun down, and both of the cells and the media were stored separately in a refrigerator until use. Amounts of cells used for each enzyme experiment were expressed as a protein concentration.

The enzyme was prepared as follows; the cells, separated from the culture medium by low speed centrifugation, were washed three times in a salined phosphate buffer solution (pH 7.0) in a cold room, and then homogenized with 4 ml of the above buffer by means of a sonicator for 30 sec. The homogenate was centrifuged at 10,000 g for 20 min,

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and the supernatant was used for enzyme assay. To the medium a crystal ammonium sulfate was added up to 0.75 saturation. The resulting precipitates were dissolved in 3 ml of the buffer solution mentioned above, and used as enzyme preparations.

Glycosidase activities were assayed in the same manner as described before (KIMURA, 1981), by the use of a *p*-nitrophenyl derivative of each glycoside as a substrate. One unit of enzyme is defined as that amount capable of liberating 1 μ mole of *p*-nitrophenol from each substrate per min at 35°C. Protein concentration was determined by Bio-Rad protein kit using a bovine serum albumin as a standard.

Some natural substances such as lactalbumin hydrolysate, TC-yeastolate, and FBS that were contained in the MM medium were assumed to have appreciable enzyme activities. Therefore, this possibility was tested for 30 ml of the fresh MM medium, the amount of which was equivalent to that of the media used for culturing cells. The results showed the existence of relatively high activity of β -*N*-acetylglucosaminidase (58 m units), and lower activity of α -mannosidase (11 m units), whereas activities of β -glucosidase and β -galactosidase (3 m units) were almost negligible. No activity of α -glucosidase was detected. Thus, all experimental values obtained from the media were corrected by subtracting the values obtained from the fresh medium.

Cell fractions from both lines exhibited extremely low and practically no activities of all enzyme tested, except for the α -glucosidase. It should be noted that the α -glucosidase in NIAS-MB-32 is two times higher than that of NIAS-MB-19 (Fig. 1A). In the medium fractions, all enzymes tested except for β -galactosidase, showed higher activities compared with those of the cell fractions. A remarkable difference between the two cell cultures was seen for both α -glucosidase and β -*N*-acetylglucosaminidase; the former enzyme was 3.6 times and the latter 1.8 fold higher in the NIAS-MB-32 than in the NIAS-MB-19. By contrast, no difference in β -glucosidase and α -mannosidase activities was present between the two culture media. β -Galactosidase was detectable neither in the NIAS-MB-32 nor NIAS-MB-19 medium (Fig. 1B).

It is apparent from the above observations that α -glucosidase and β -*N*-acetylglucosaminidase are primarily synthesized by the cells and immediately

Fig. 1. Glycosidase activities of two cell lines from the ovary of *Mamestra brassicae*. A, cells (protein concentration in cells of NIAS-MB-19 and NIAS-MB-32 were 3.3 mg and 4.3 mg respectively); B, MM medium used for culturing cells for a week (30 ml). Hollow bar, NIAS-MB-19 cell line; hatched bar, NIAS-MB-32 cell line. α -Gluc, α -glucosidase; β -Gluc, β -glucosidase; β -N-AcG, β -*N*-acetylglucosaminidase; α -Man, α -mannosidase; β -Gal, β -galactosidase.

released into the culture medium. However, it remains in question whether the same interpretation is applicable to β -glucosidase and α -mannosidase, because their activities are too low. The above observations also suggest the possibility that the two cell lines can be distinguishable from each other by biochemical means, e.g. by comparison of enzyme activity, even though these cell lines were of homologous origin. It is particularly interesting that the cell lines derived from ovaries exhibit the activity of the α -glucosidase, which is known to be involved in the regulation of glucose metabolism in insects (MATTHEWS et al., 1976). DZIADIK-TURNER et al. (1981) has demonstrated β -*N*-acetylglucosaminidase in the media of the cell lines from *Culex quinquefasciatus* (ovary) and *Drosophila melanogaster* (embryo) but in neither the medium of *Aedes pseudoscutellaris* (ovary) nor *Trichoplusia ni* (ovary) cells. The physiological function of this enzyme in the reproductive system of insects is still unknown, but it is assumed to function is glycoprotein metabolism as reported for mammalian system.

β -Galactosidase has extensively been investigated in *Drosophila* cell lines from embryos in connection with the mechanism by which an insect hormone induces this enzyme activity (BEST-BELPOMME et al., 1978). It may be, therefore, worthy to examine whether this enzyme activity is also induced in

ovarian cell lines by application of some ecdysteroids.

It can be said from the present experiment that an insect ovarian cell line produces a considerable amount of α -glucosidase and β -N-acetylglucosaminidase. In this connection, however, a possibility that the zymogen in the fetal bovine serum is activated by insect cells and exhibits the enzyme activity, cannot be excluded. Further experiments will be necessary to resolve this problem.

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The Intracellular Yeastlike Symbiotes of *Laodelphax striatellus* FALLÉN (Homoptera: Delphacidae) Strains Resistant and Susceptible to Malathion^{1,2}

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Many insect species possess internal flora of microorganisms (BUCHNER, 1965). An intracellular symbiote found in *Laodelphax striatellus* FALLÉN has been reported to play an important role at least in supplying sufficient sterol for the development of *L. striatellus* (NODA and SAITO, 1979 b). It was reported that the lengths of individual symbiotes and the number of these per unit area in ultra thin sections of mycetomes did not differ significantly from strains of *Myzus persicae* SULZ. resistant and susceptible to demeton-S-methyl (BALL and BAILEY, 1978). On the other hand, contrary observations were reported in strains of *M. persicae* susceptible and resistant to parathion (AMIRESSAMI and PETZOLD, 1976/1977). In this paper the relationship between the intracellular yeastlike symbiote

and malathion resistance of *L. striatellus* was studied.

L. striatellus, collected at Kitashirakawa, Kyoto in 1965, was reared on rice seedlings at 25°C under a 16-hr light, 8-hr dark cycle. This population was used as a susceptible (S) strain. The malathion resistant (Rm) strain was obtained by selecting the S strain with malathion (OZAKI and KASSAI, 1971). Heat treatment was conducted by the exposure of insect after hatching to 35°C for 3 days to obtain *L. striatellus* populations with a small number of the yeastlike symbiotes (NODA and SAITO, 1979 a). The number of the yeastlike symbiotes was counted by means of a THOMA's hemocytometer (NODA, 1974). Susceptibility of *L. striatellus* to malathion was measured by means of a dry film method (OZAKI and KASSAI, 1971). LD₅₀ values were calculated according to BLISS (1935). *In vitro* degradation of ¹⁴C-methyl malathion by *L. striatellus* homogenates was studied by incubating ¹⁴C-methyl malathion with the homogenates at 37°C for 30 min (MIYATA et al., 1976).

The results are shown in Table 1. The Rm strain showed a higher resistance level to malathion than the S strain. *In vitro* ¹⁴C-methyl malathion degradation activity was also higher in the Rm strain than in the S strain as reported previously (MIYATA et al., 1976). There was no significant difference in the number of the yeastlike symbiotes between the S and the Rm strains.

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